PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/54, 9/10, 15/81, 15/82, 1/16, 5/10, A01N 27/067, C12P 7/64

A2

(11) International Publication Number: WO 00/60095

(43) International Publication Date: 12 October 2000 (12.10.00)

(21) International Application Number: PCT/EP00/02701

(22) International Filing Date: 28 March 2000 (28.03.00)

(30) Priority Data:

99106656.4	1 April 1999 (01.04.99)	EP
99111321.8	10 June 1999 (10.06.99)	EP
60/180,687	7 February 2000 (07.02.00)	US

(71) Applicant *(for all designated States except US)*: BASF PLANT SCIENCE GMBH [DE/DE]; D–67056 Ludwigshafen (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: DAHLQVIST, Anders [SE/SE]; Hemmansvägen 2, S–244 66 Furulund (SE). STAHL, Ulf [SE/SE]; Liljegatan 7b, S–753 24 Uppsala (SE). LENMAN, Marit [SE/SE]; Revingegatan 13a, S–223 59 Lund (SE). BANAS, Antoni [SE/PL]; Wiolinowa 14, PL–08110 Siedlce (PL). RONNE, Hans [SE/SE]; Dirigentvägen 169, S–756 54 Uppsala (SE). STYMNE, Sten [SE/SE]; Torrlösa 1380, S–269 90 Svalöv (SE).

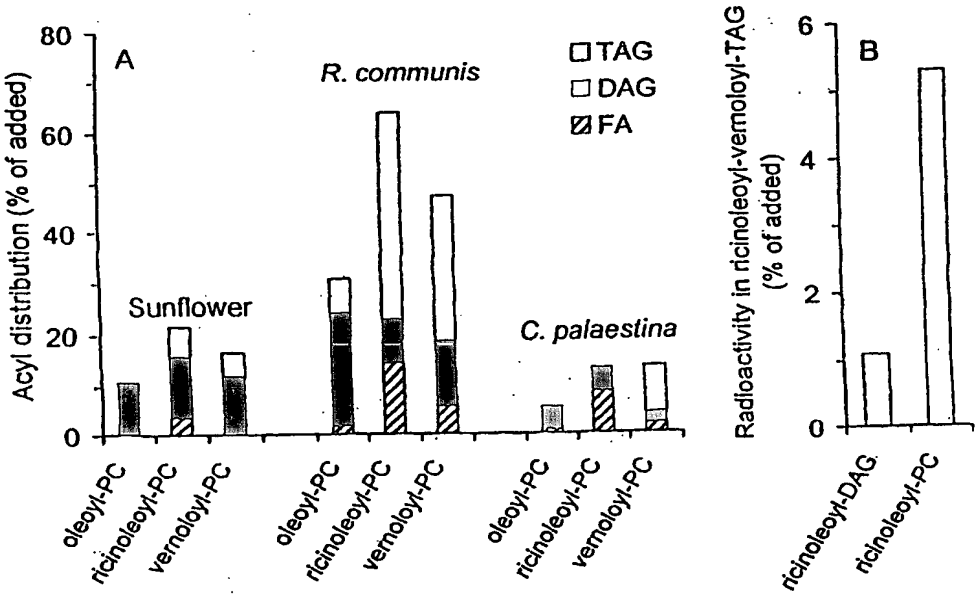
(74) Agent: FITZNER, Uwe; Lintorfer Str. 10, D–40878 Ratingen (DE).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: A NEW CLASS OF ENZYMES IN THE BIOSYNTHETIC PATHWAY FOR THE PRODUCTION OF TRIACYLGLYCEROL AND RECOMBINANT DNA MOLECULES ENCODING THESE ENZYMES



(57) Abstract

The present invention relates to the isolation, identification and characterization of nucleotide sequences encoding an enzyme catalysing the transfer of fatty acids from phospholipids to diacylglycerol in the biosynthetic pathway for the production of triacylglycerol, to the said enzymes and a process for the production of triacylglycerols.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

A NEW CLASS OF ENZYMES IN THE BIOSYNTHETIC PATHWAY FOR THE PRODUCTION OF TRIACYLGLYCEROL AND RECOMBINANT DNA MOLECULES ENCODING THESE ENZYMES

The present invention relates to the isolation, identification and characterization of recombinant DNA molecules encoding enzymes catalysing the transfer of fatty acids from phospholipids to diacylglycerol in the biosynthetic pathway for the production of triacylglycerol.

Triacylglycerol (TAG) is the most common lipid-based energy reserve in nature. The main pathway for synthesis of TAG is believed to involve three sequential acyl-transfers from acyl-CoA to a glycerol backbone (1, 2). For many years, acyl-CoA : diacylglycerol acyltransferase (DAGAT), which catalyzes the third acyl transfer reaction, was thought to be the only unique enzyme involved in TAG synthesis. It acts by diverting diacylglycerol (DAG) from membrane lipid synthesis into TAG (2). Genes encoding this enzyme were recently identified both in the mouse (3) and in plants (4, 5), and the encoded proteins were shown to be homologous to acyl-CoA : cholesterol acyltransferase (ACAT). It was also recently reported that another DAGAT exists in the oleaginous fungus *Mortierella ramanniana,* which is unrelated to the mouse DAGAT, the ACAT gene family or to any other known gene (6).

The instant invention relates to novel type of enzymes and their encoding genes for transformation. More specifically, the invention relates to use of a type of genes encoding a not previously described type of enzymes hereinafter designated phospholipid:diacylglycerol acyltransferases (PDAT), whereby this enzyme catalyses an acyl-CoA-independent reaction. The said type of genes expressed alone in transgenic organisms will enhance the total amount of oil (triacylglycerols) produced in the cells. The PDAT genes, in combination with a gene for the synthesis of an uncommon fatty acid will, when expressed in transgenic organisms, enhance the levels of the uncommon fatty acids in the triacylglycerols.

There is considerable interest world-wide in producing chemical feedstock, such as fatty acids, for industrial use from renewable plant resources rather than non-renewable petrochemicals. This concept has broad appeal to manufacturers and consumers on the basis of resource conservation and provides significant opportunity to develop new industrial crops for agriculture.

There is a diverse array of unusual fatty acids in oils from wild plant species and these have been well characterised. Many of these acids have industrial potential and this has led to interest in domesticating relevant plant species to enable agricultural production of particular fatty acids.

Development in genetic engineering technologies combined with greater understanding of the biosynthesis of unusual fatty acids now makes it possible to transfer genes coding for key enzymes involved in the synthesis of a particular fatty acid from a wild species into domesticated oilseed crops. In this way individual fatty acids can be produced in high purity and quantities at moderate costs.

In all crops like rape, sunflower, oilpalm etc., the oil (i.e. triacylglycerols) is the most valuable product of the seeds or fruits and other compounds like starch, protein, and fibre is regarded as by-products with less value. Enhancing the quantity of oil per weight basis at the expense of other compounds in oil crops would therefore increase the value of crop. If genes, regulating the allocation of reduced carbon into the production of oil can be up-regulated, the cells will accumulate more oil on the expense of other products. Such genes might not only be used in already high oil producing cells, such as oil crops, but could also induce significant oil production in moderate or low oil containing crops such as e.g. soy, oat, maize, potato, sugarbeats, and turnips as well as in micro-organisms.

Summary of the invention

Many of the unusual fatty acids of interest, e.g. medium chain fatty acids, hydroxy fatty acids, epoxy fatty acids and acetylenic fatty acids, have physical properties that are distinctly different from the common plant fatty acids. The present inventors have found that, in plant species naturally accumulating these uncommon fatty acids in their seed oil (i.e. triacylglycerol), these acids are absent, or present in very low amounts in the membrane (phospho)lipids of the seed. The low concentration of these acids in the membrane lipids is most likely a prerequisite for proper membrane function and thereby for proper cell functions. One aspect of the invention is that seeds of transgenic crops can be made to accumulate high amounts of uncommon fatty acids if these fatty acids are efficiently removed from the membrane lipids and channelled into seed triacylglycerols.

The inventors have identified a novel class of enzymes in plants catalysing the transfer of fatty acids from phospholipids to diacylglycerol in the production of triacylglycerol through an acyl-CoA-independent reaction and that these enzymes (phospholipid:diacylglycerol acyltransferases, abbreviated as PDAT) are involved in the removal of hydroxylated, epoxygenated fatty acids, and probably also other uncommon fatty acids such as medium chain fatty acids, from phospholipids in plants.

This enzyme reaction was shown to be present in microsomal preparations from baker's yeast (*Saccharomyces cerevisiae*). The instant invention further pertains to an enzyme comprising an amino acid sequence as set forth in SEQ ID No. 2 or a functional fragment, derivate, allele, homolog or isoenzyme thereof. A so called ‚knock out' yeast mutant, disrupted in the respective gene was obtained and microsomal membranes from the mutant was shown to totally lack PDAT activity. Thus, it was proved that the disrupted gene encodes a PDAT enzyme (SEQ ID NO. 1 and 2). Furtherm, this PDAT enzyme is

characterized through the amino acid sequence as set forth in SEQ ID NO 2 containing a lipase motif of the conserved sequence string FXKWVEA.

The instant invention pertains further to an enzyme comprising an amino acid sequence as set forth in SEQ ID NO. 1a, 2b or 5a or a functional fragment, derivate, allele, homolog or isoenzyme thereof.

Further genes and/or proteins of so far unknown function were identified and are contemplated within the scope of the instant invention. A gene from Schizosaccharomyces pombe, SPBC776.14 (SEQ ID. NO. 3), a putative open reading frame CAA22887 of the SPBC776.14 (SEQ ID NO. 13) were identified. Further Arabidopsis thaliana genomic sequences (SEQ ID NO. 4, 10 and 11) coding for putative proteins were identified, as well as a putative open reading frame AAC80628 from the A. thaliana locus AC 004557 (SEQ ID NO. 14) and a putative open reading frame AAD10668 from the A. thaliana locus AC 003027 (SEQ ID NO. 15) were identified.

Also, a partially sequenced cDNA clone from Neurospora crassa (SEQ ID NO. 9) and a Zea mays EST (Extended Sequence Tac) clone (SEQ ID NO. 7) and corresponding putative amino acid sequence (SEQ ID NO. 8) were identified. Finally, two cDNA clones were identified, one Arabidopsis thaliana EST (SEQ ID NO. 5 and corresponding predicted amino acid sequence SEQ ID NO. 6) and a Lycopersicon esculentum EST clone (SEQ ID NO. 12) were identified.

Further, enzymes designated as PDAT comprising an amino acid sequence selected from the group consisting of sequences as set forth in SEQ ID NO 2a, 3a, 5b, 6 or 7b containing a lipase motif FXKWVEA are contemplated within the scope of the invention. Moreover, an enzyme comprising an amino acid sequence encoded through a nucleotide sequence, a portion, derivate, allele or homolog thereof selected from the group consisting of sequences as set forth in SEQ ID No. 1, 1b, 3, 3b, 4, 4a, 4b, 5, 5b, 6b, 7, 8b, 9, 9b, 10, 10b, 11, 11b or 12 or a functional fragment, derivate, allele, homolog or isoenzyme of the enzyme encoding amino acid sequence are included within the scope of the invention.

A functional fragment of the instant enzyme is understood to be any polypeptide sequence which shows specific enzyme activity of a phospholipid:diacylglycerol acyltransferase (PDAT). The length of the functional fragment can for example vary in a range from about 660 ± 10 amino acids to 660 ± 250 amino acids, preferably from about 660 ± 50 to 660 ±100 amino acids, whereby the „basic number" of 660 amino acids corresponds in this case to the polypeptide chain of the PDAT enzyme of SEQ ID NO. 2 encoded by a nucleotide sequence according to SEQ ID NO. 1. Consequently, the „basic number" of functional fullength enzyme can vary in correspondance to the encoding nucleotide sequence.

A portion of the instant nucleotide sequence is meant to be any nucleotide sequence encoding a polypeptid which shows specific activity of a phospholipid:diacylglycerol acyltransferase (PDAT). The length of the nucleotide portion can vary in a wide range of about several hundreds of nucleotides based upon the coding region of the gene or a highly conserved sequence. For example the length varies in a range form about 1900 ± 10 to 1900 ± 1000 nucleotides, preferably form about 1900 ± 50 to 1900 ±700 and more preferably form about 1900 ± 100 to 1900 ± 500 nucleotides. whereby the „basic number" of 1900 nucleotides corresponds in this case to the encoding nucleotide sequence of the PDAT enzyme of SEQ ID NO. 1. Consequently, the „basic number" of functional fullength gene can vary.

An allelic variant of the instant nucleotide sequence is understood to be any different nucleotide sequence which encodes a polypeptide with a functionally equivalent function. The alleles pertain naturally occuring variants of the instant nucleotide sequences as well as synthetic nucleotide sequences produced by methods known in the art. Contemplated are even altered nucleotide sequences which result in an enzyme with altered activity and/or regulation or which is resistant against specific inhibitors. The instant invention further includes natural or synthetic mutations of the originally isolated nucleotide

sequences. These mutations can be substitution, addition, deletion, inversion or insertion of one or more nucleotides.

A homologous nucleotide sequence is understood to be a complementary sequence and/or a sequence which specifically hybridizes with the instant nucleotide sequence. Hybridizing sequences include similar sequences selected from the group of DNA or RNA which specifically interact to the instant nucleotide sequences under at least moderate stringency conditions which are known in the art. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C. This further includes short nucleotide sequences of e.g. 10 to 30 nucleotides, preferably 12 to 15 nucleotides. Included are also primer or hybridization probes.

A homologous nucleotide sequence included within the scope of the instant invention is a sequence which is at least about 40%, preferably at least about 50 % or 60%, and more preferably at least about 70%, 80% or 90% and most preferably at least about 95%, 96%, 97%, 98% or 99% or more homologous to a nucleotide sequence of SEQ ID NO. 1.
All of the aforementioned definitions are true for amino acid seqences and functional enzymes and can easily transfered by a person skilled in the art.

Isoenzymes are understood to be enzymes which have the same or a similar substrate specifity and/or catalytic activity but a different primary structure.

In a first embodiment, this invention is directed to nucleic acid sequences that encode a PDAT. This includes sequences that encode biologically active PDATs as well as sequences that are to be used as probes, vectors for transformation or cloning intermediates. The PDAT encoding sequence may

encode a complete or partial sequence depending upon the intended use. All or a portion of the genomic sequence, cDNA sequence, precursor PDAT or mature PDAT is intended.

Further included is a nucleotide sequence selected from the group consisting of sequences set forth in SEQ ID No. 1, 1b, 3, 3b, 4, 4a, 4b, 9b, 10, 10b or 11 or a portion, derivate, allele or homolog thereof. The invention pertains a partial nucleotide sequence corresponding to a fullength nucleotide sequence selected from the group consisting of sequences set forth in SEQ ID No. 5, 5b, 6b, 7, 8b, 9, 11b or 12 or a portion, derivate, allele or homolog thereof. Moreover, a nucleotide sequence comprising a nucleotide sequence which is at least 40% homologous to a nucleotide sequence selected form the group consisting of those sequences set forth in SEQ ID No. 1 1b, 3, 3b, 4, 4a, 4b, 5, 5b, 6b, 7, 8b, 9, 9b, 10, 10b, 11, 11b or 12 is contemplated within the scope of the invention.

The instant invention pertains to a gene construct comprising a said nucleotide sequences of the instant invention which is operably linked to a heterologous nucleic acid.
The term operably linked means a serial organisation e.g. of a promotor, coding sequence, terminator and/or further regulatory elements whereby each element can fulfill its original function during expression of the nucleotide sequence.

Further, a vector comprising of a said nucleotide sequence of the instant invention is contemplated in the instant invention. This includes also an expression vector as well as a vector further comprising a selectable marker gene and/or nucleotide sequences for the replication in a host cell and/or the integration into the genome of the host cell.

In a different aspect, this invention relates to a method for producing a PDAT in a host cell or progeny thereof, including genetically engineered oil seeds, yeast and moulds or any other oil accumulating organism, via the expression of a

construct in the cell. Cells containing a PDAT as a result of the production of the PDAT encoding sequence are also contemplated within the scope of the invention.

Further, the invention pertains a transgenic cell or organism containing a said nucleotide sequence and/or a said gene construct and/or a said vector. The object of the instant invention is further a transgenic cell or organism which is an eucaryotic cell or organism. Preferably, the transgenic cell or organism is a yeast cell or a plant cell or a plant. The instant invention further pertains said transgenic cell or organism having an altered biosynthetic pathway for the production of triacylglycerol. A transgenic cell or organism having an altered oil content is also contemplated within the scope of this invention.

Further, the invention pertains a transgenic cell or organism wherein the activity of PDAT is altered in said cell or organism. This altered activity of PDAT is characterized by an alteration in gene expression, catalytic activity and/or regulation of activity of the enzyme. Moreover, a transgenic cell or organism is included in the instant invention, wherein the altered biosynthetic pathway for the production of triacylglycerol is characterized by the prevention of accumulation of undesirable fatty acids in the membrane lipids.

In a different embodiment, this invention also relates to methods of using a DNA sequence encoding a PDAT for increasing the oil-content within a cell.

Another aspect of the invention relates to the accommodation of high amounts of uncomman fatty acids in the triacylglycerol produced within a cell, by introducing a DNA sequence producing a PDAT that specifically removes these fatty acids from the membrane lipids of the cell and channel them into triacylglycerol. Plant cells having such a modification are also contemplated herein.

Further, the invention pertains a process for the production of triacylglycerol, comprising growing a said transgenic cell or organism under conditions whereby the said nucleotide sequence is expressed and whereby the said transgenic cells comprising a said enzyme catalysing the transfer of fatty acids from phospholipids to diacylglycerol forming triacylglycerol.

Moreover, triacylglycerols produced by the aforementioned process are included in scope of the instant invention.

Object of the instant invention is further the use of an instant nucleotide sequence and/or a said enzyme for the production of triacylglycerol and/or triacylglycerols with uncommon fatty acids. The use of a said instant nucleotide sequence and/or a said enzyme of the instant invention for the transformation of any cell or organism in order to be expressed in this cell or organism and result in an altered, preferably increased oil content of this cell or organism is also contemplated within the scope of the instant invention.

A PDAT of this invention includes any sequence of amino acids, such as a protein, polypeptide or peptide fragment obtainable from a microorganism, animal or plant source that demonstrates the ability to catalyse the production of triacylglycerol from a phospholipid and diacylglycerol under enzyme reactive conditions. By „enzyme reactive conditions" is meant that any necessary conditions are available in an environment (e.g., such factors as temperature, pH, lack of inhibiting substances) which will permit the enzyme to function.

Other PDATs are obtainable from the specific sequences provided herein. Furthermore, it will be apparent that one can obtain natural and synthetic PDATs, including modified amino acid sequences and starting materials for synthetic-protein modelling from the examplified PDATs and from PDATs which are obtained through the use of such examplified sequences. Modified amino acid sequences include sequences that have been mutated, truncated,

increased and the like, whether such sequences were partially or wholly synthesised. Sequences that are actually purified from plant preparations or are identical or encode identical proteins thereto, regardless of the method used to obtain the protein or sequence, are equally considered naturally derived.

Further, the nucleic acid probes (DNA and RNA) of the present invention can be used to screen and recover „homologous" or „related" PDATs from a variety of plant and microbial sources.

Further, it is also apparent that a person skilled in the art can, with the information provided in this application, in any organism identify a PDAT activity, purify an enzyme with this activity and thereby identify a „non-homologous" nucleic acid sequence encoding such an enzyme.

The present invention can be essentially characterized by the following aspects:

1. Use of a PDAT gene (genomic clone or cDNA) for transformation.
2. Use of a DNA molecule according to item 1 wherein said DNA is used for transformation of any organism in order to be expressed in this organism and result in an active recombinant PDAT enzyme in order to increase oil content of the organism.
3. Use of a DNA molecule of item 1 wherein said DNA is used for transformation of any organism in order to prevent the accumulation of undesirable fatty acids in the membrane lipids.
4. Use according to item 1, wherein said PDAT gene is used for transforming transgenic oil accumulating organisms engineered to produce any uncommon fatty acid which is harmful if present in high amounts in membrane lipids, such as medium chain fatty acids, hydroxylated fatty acids, epoxygenated fatty acids and acetylenic fatty acids.

5. Use according to item 1, wherein said PDAT gene is used for transforming organisms, and wherein said organisms are crossed with other oil accummulating organisms engineered to produce any uncommon fatty acid which is harmful if present in high amounts in membrane lipids, comprising medium chain fatty acids, hydroxylated fatty acids, epoxygenated fatty acids and acetylenic fatty acids.
6. Use according to item 1, wherein the enzyme encoded by said PDAT gene or cDNA is coding for a PDAT with distinct acyl specificity.
7. Use according to item 1 wherein said PDAT encoding gene or cDNA, is derived from *Saccharomyces cereviseae,* or contain nucleotide sequences coding for an amino acid sequence 30% or more identical to the amino acid sequence of PDAT as presented in SEQ. ID. NO. 2.
8. Use according to item 1 wherein said PDAT encoding gene or cDNA is derived from *Saccharomyces cereviseae,* or contain nucleotide sequences coding for an amino acid sequence 40% or more *identical* to the amino acid sequence of PDAT as presented in SEQ. ID. NO. 2.
9. Use according to item 1 wherein said PDAT encoding gene or cDNA is derived from *Saccharomyces cereviseae,* or contain nucleotide sequences coding for an amino acid sequence 60% or more *identical* to the amino acid sequence of PDAT as presented in SEQ. ID. NO. 2.
10. Use according to item 1 wherein said PDAT encoding gene or cDNA is derived from *Saccharomyces cereviseae,* or contain nucleotide sequences coding for an amino acid sequence 80% or more identical to the amino acid sequence of PDAT as presented in SEQ. ID. NO. 2.
11. Use according to item 1 wherein said PDAT encoding gene or cDNA is derived from plants or contain nucleotide sequences coding for an amino acid sequence 40% or more identical to the amino acid sequence of PDAT from *Arabidopsis thaliana* or to the protein encoded by the fullength counterpart of the partial Zea mays, Lycopericon esculentum, or Neurospora crassa cDNA clones.

12. Transgenic oil accumulating organisms comprising, in their genome, a PDAT gene transferred by recombinant DNA technology or somatic hybridization.
13. Transgenic oil accumulating organisms according to item 12 comprising, in their genome, a PDAT gene having specificity for substrates with a particular uncommon fatty acid and the gene for said uncommon fatty acid.
14. Transgenic organisms according to item 12 or 13 which are selected from the group consisting of fungi, plants and animals.
15. Transgenic organisms according to item 12 or 13 which are selected from the group of agricultural plants.
16. Transgenic organisms according to item 12 or 13 which are selected from the group of agricultural plants and where said PDAT gene is expressed under the control of a storage organ specific promotor.
17. Transgenic organisms according to item 12 or 13 which are selected from the group of agricultural plants and where said PDAT gene is expressed under the control of a seed promotor.
18. Oils from organisms according to item 12 – 17.
19. A method for altering acyl specificity of a PDAT by alteration of the nucleotide sequence of a naturally occurring encoding gene and as a consequence of this alternation creating a gene encoding for an enzyme with novel acyl specifity.
20. A protein encoded by a DNA molecule according to item 1 or a functional fragment thereof.
21. A protein of item 20 designated phospholipid:diacylglycerol acyltransferase.
22. A protein of item 21 which has a distinct acyl specificity.
23. A protein of item 13 having the amino acid sequence as set forth in SEQ, ID NO. 2, 13, 14 or 15 (and the proteins encoded by the fullength or partial genes set forth in SEQ. ID. NO. 1, 3, 4, 5, 7, 9, 10, 11 or 12) or an amino acid sequence with at least 30 % homology to said amino acid sequence.
24. A protein of item 23 isolated from Saccharomyces cereviseae.

General methods:

Yeast strains and plasmids. The wild type yeast strains used were either FY1679 (*MATα his3-Δ200 leu2-Δ1 trp1-Δ6 ura3-52*) or W303-1A (*MATa ADE2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*) (7). The YNR008w::KanMX2 disruption strain FVKT004-04C(AL), which is congenic to FY1679, was obtained from the Euroscarf collection (8). A 2751 bp fragment containing the YNR008w gene with 583 bp of 5' and 183 bp of 3' flanking DNA was amplified from W303-1A genomic DNA using *Taq* polymerase with 5'-TCTCCATCTTCTGCAAAACCT-3' and 5'-CCTGTCAAAAACCTTCTCCTC-3' as primers. The resulting PCR product was purified by agarose gel electrophoresis and cloned into the *Eco*RV site of pBluescript (pbluescript-pdat). For complementation experiments, the cloned fragment was released from pBluescript by *Hin*dIII-*Sac*I digestion and then cloned between the *Hin*dIII and *Sac*I sites of pFL39 (9), thus generating pUS1. For overexpression of the PDAT gene, a 2202 bp *Eco*RI fragment from the pBluscript plasmid which contains only 24 bp of 5' flanking DNA was cloned into the BamHI site of the *GAL1-TPK2* expression vector pJN92 (12), thus generating pUS4.

Microsomal preparations. Microsomes from developing seeds of sunflower (*Helianthus annuus*), *Ricinus communis* and *Crepis palaestina* were prepared using the procedure of Stobart and Stymne (11). To obtain yeast microsomes, 1g of yeast cells (fresh weight) was re-suspended in 8 ml of ice-cold buffer (20 mM Tris-Cl, pH 7.9, 10 mM $MgCl_2$, 1 mM EDTA, 5 % (v/v) glycerol, 1 mM DTT, 0.3 M ammonium sulfate) in a 12 ml glass tube. To this tube, 4 ml of glass beads (diameter 0.45-0.5 mm) were added, and the tube was then heavily shaken (3 x 60 s) in an MSK cell homogenizer (B. Braun Melsungen AG, Germany). The homogenized suspension was centrifuged at 20,000 x g for 15 min at 6°C and the resulting supernatant was again centrifuged at 100,000 x g for 2 h at 6°C. The 100,000 x g pellet was resuspended in 0.1 M potassium

phosphate (pH 7.2), and stored at -80°C. It is subsequently referred to as the crude yeast microsomal fraction.

Lipid substrates. Radio-labeled ricinoleic (12-hydroxy-9-octadecenoic) and vernolic (12,13-epoxy-9-octadecenoic) acids were synthesized enzymatically from [1-^{14}C]oleic acid and [1-^{14}C]linoleic acid, respectively, by incubation with microsomal preparations from seeds of *Ricinus communis* and *Crepis palaestina*, respectively (12). The synthesis of phosphatidylcholines (PC) or phosphatidylethanolamines (PE) with ^{14}C-labeled acyl groups in the *sn*-2 position was performed using either enzymatic (13), or synthetic (14) acylation of [^{14}C]oleic, [^{14}C]ricinoleic, or [^{14}C]vernolic acid. Dioleoyl-PC that was labeled in the *sn*-1 position was synthesized from *sn*-1-[^{14}C]oleoyl-lyso-PC and unlabeled oleic acid as described in (14). *Sn*-1-oleoyl-*sn*-2-[^{14}C]ricinoleoyl-DAG was synthesized from PC by the action of phospholipase C type XI from *B. Cereus* (Sigma Chemical Co.) as described in (15). Monovernoloyl- and divernoleoyl-DAG were synthesized from TAG extracted from seeds of *Euphorbia lagascae*, using the TAG-lipase (Rizhopus arrhizus, Sigma Chemical Co.) as previously described (16). Monoricinoleoyl-TAG was synthesized according to the same method using TAG extracted from Castor bean.

Lipid analysis. Total lipid composition of yeast were determined from cells harvested from a 40 ml liquid culture, broken in a glass-bead shaker and extracted into chloroform as described by Bligh and Dyer (17), and then separated by thin layer chromatography in hexane/diethylether/acetic acid (80:20:1) using pre-coated silica gel 60 plates (Merck). The lipid areas were located by brief exposure to I_2 vapors and identified by means of appropriate standards. Polar lipids, sterol-esters and triacylglycerols, as well as the remaining minor lipid classes, referred to as other lipids, were excised from the plates. Fatty acid methylesters were prepared by heating the dry excised material at 85 °C for 60 min in 2% (v/v) sulfuric acid in dry methanol. The methyl esters were extracted with hexane and analyzed by GLC through a 50 m

x 0.32 mm CP-Wax58-CB fused-silica column (Chrompack), with methylheptadecanoic acid as an internal standard. The fatty acid content of each fraction was quantified and used to calculate the relative amount of each lipid class. In order to determine the total lipid content, 3 ml aliquots from yeast cultures were harvested by centrifugation and the resulting pellets were washed with distilled water and lyophilized. The weight of the dried cells was determined and the fatty acid content was quantified by GLC-analyses after conversion to methylesters as described above. The lipid content was then calculated as nmol fatty acid (FA) per mg dry weight yeast.

Enzyme assays. Aliquots of crude microsomal fractions (corresponding to 10 nmol of microsomal PC) from developing plant seeds or yeast cells were lyophilized over night. ^{14}C-Labeled substrate lipids dissolved in benzene were then added to the dried microsomes. The benzene was evaporated under a stream of N_2, leaving the lipids in direct contact with the membranes, and 0.1 ml of 50 mM potassium phosphate (pH 7.2) was added. The suspension was thoroughly mixed and incubated at 30°C for the time period indicated, up to 90 min. Lipids were extracted from the reaction mixture using chloroform and separated by thin layer chromatography in hexane/diethylether/acetic acid (35:70:1.5) using silica gel 60 plates (Merck). The radioactive lipids were visualized and quantified on the plates by electronic autoradiography (Instant Imager, Packard, US).

Yeast cultivation. Yeast cells were grown at 28°C on a rotatory shaker in liquid YPD medium (1% yeast extract, 2% peptone, 2% glucose), synthetic medium (18) containing 2% (v/v) glycerol and 2% (v/v) ethanol, or minimal medium (19) containing 16 g/l of glycerol.

The instant invention is further characterized by the following examples which are not limiting:

Acyl-CoA-independent synthesis of TAG by oil seed microsomes. A large number of unusual fatty acids can be found in oil seeds (20). Many of these fatty acids, such as ricinoleic (21) and vernolic acids (22), are synthesized using phosphatidylcholin (PC) with oleoyl or linoleoyl groups esterified to the *sn*-2 position, respectively, as the immediate precursor. However, even though PC can be a substrate for unusual fatty acid synthesis and is the major membrane lipids in seeds, unusual fatty acids are rarely found in the membranes. Instead, they are mainly incorporated into the TAG. A mechanism for efficient and selective transfer of these unusual acyl groups from PC into TAG must therefore exist in oil seeds that accumulate such unusual fatty acids. This transfer reaction was biochemically characterized in seeds from castor bean (*Ricinus communis)* and *Crepis palaestina,* plants which accumulate high levels of ricinoleic and vernolic acid, respectively, and sunflower (*Helianthus annuus)*, a plant which has only common fatty acids in its seed oil. Crude microsomal fractions from developing seeds were incubated with PC having ^{14}C-labeled oleoyl, ricinoleoyl or vernoloyl groups at the *sn*-2 position. After the incubation, lipids were extracted and analyzed by thin layer chromatography. We found that the amount of radioactivity that was incorporated into the neutral lipid fraction increased linearly over a period of 4 hours (data not shown). The distribution of [^{14}C]acyl groups within the neutral lipid fraction was analyzed after 80 min (Fig. 1). Interestingly the amount and distribution of radioactivity between diffferent neutral lipids were strongly dependent both on the plant species and on the type of [^{14}C]acyl chain. Thus, sunflower microsomes incorporated most of the label into DAG, regardless of the type of [^{14}C]acyl group. In contrast, *R. communis* microsomes preferentially incorporated [^{14}C]ricinoleoyl and [^{14}C]vernoloyl groups into TAG, while [^{14}C]oleyl groups mostly were found in DAG. *C. palaestina* microsomes, finally, incorporated only [^{14}C]vernolyol groups into TAG, with [^{14}C]ricinoleyl groups being found mostly as free fatty acids, and [^{14}C]oleyl groups in DAG. This shows that the high *in vivo* levels of ricinoleic acid and vernolic acid in the TAG pool of *R. communis*

and *C. palaestina*, respectively, can be explained by an efficient and selective transfer of the corresponding acyl groups from PC to TAG in these organisms.

The in-vitro synthesis of triacylglycerols in microsomal preparations of developing castor bean is summarized in table 1.

PDAT: a novel enzyme that catalyzes acyl-CoA independent synthesis of TAG. It was investigated if DAG could serve both as an acyl donor as well as an acyl acceptor in the reactions catalyzed by the oil seed microsomes. Thererfore, unlabeled divernoloyl-DAG was incubated with either *sn*-1-oleoyl-*sn*-2-[^{14}C]ricinoleoyl-DAG or *sn*-1-oleoyl-*sn*-2-[^{14}C]ricinoleoyl-PC in the presence of *R. communis* microsomes. The synthesis of TAG molecules containing both [^{14}C]ricinoleoyl and vernoloyl groups was 5 fold higher when [^{14}C]ricinoleoyl-PC served as acyl donor as compared to [^{14}C]ricinoleoyl-DAG (fig.1B). These data strongly suggests that PC is the immediate acyl donor and DAG the acyl acceptor in the acyl-CoA-independent formation of TAG by oil seed microsomes. Therefore, this reaction is catalyzed by a new enzyme which we call phospholipid : diacylglycerol acyltransferase (PDAT).

PDAT activity in yeast microsomes. Wild type yeast cells were cultivated under conditions where TAG synthesis is induced. Microsomal membranes were prepared from these cells and incubated with *sn*-2-[^{14}C]-ricinoleoyl-PC and DAG and the ^{14}C-labeled products formed were analyzed. The PC-derived [^{14}C]ricinoleoyl groups within the neutral lipid fraction mainly were found in free fatty acids or TAG, and also that the amount of TAG synthesized was dependent on the amount of DAG that was added to the reaction (Fig.2). The *in vitro* synthesis of TAG containing both ricinoleoyl and vernoloyl groups, a TAG species not present *in vivo*, from exogenous added *sn*-2-[^{14}C]ricinoleoyl-PC and unlabelled vernoloyl-DAG (Fig. 2, lane 3) clearly demonstrates the existence of an acyl-CoA-independent synthesis of TAG involving PC and DAG as

substrates in yeast microsomal membranes. Consequently, TAG synthesis in yeast can be catalyzed by an enzyme similar to the PDAT found in plants.

The PDAT encoding gene in yeast.

A gene in the yeast genome (YNR008w) is known, but nothing is known about the function of YNR008w, except that the gene is not essential for growth under normal circumstances. Microsomal membranes were prepared from the yeast strain FVKT004-04C(AL) (8) in which this gene with unknown function had been disrupted. PDAT activity in the microsomes were assayed using PC with radiolabelled fatty acids at the sn-2 position. The activity was found to be completely absent in the disruption strain (Fig. 2 lane 4). Significantly, the activity could be partially restored by the presence of YNR008w on the single copy plasmid pUS1 (Fig. 2 lane 5). Moreover, acyl groups of phosphatidylethanolamine (PE) were efficiently incorporated into TAG by microsomes from the wild type strain whereas no incorporation occured from this substrate in the mutant strain (data not shown). This shows that YNR008w encodes a yeast PDAT which catalyzes the transfer of an acyl group from the *sn*-2 position of phospholipids to DAG, thus forming TAG. It should be noted that no cholesterol esters were formed from radioactive PC even in incubations with added ergosterols, nor were the amount of radioactive free fatty acids formed from PC affected by disruption of the YNR008w gene (data not shown). This demonstrates that yeast PDAT do not have cholesterol ester synthesising or phospholipase activities.

Increased TAG content in yeast cells that overexpress PDAT. The effect of overexpressing the PDAT-encoding gene was studied by transforming a wild type yeast strain with the pUS4 plasmid in which the gene is expressed from the galactose-induced *GAL1:TPK2* promoter. Cells containing the empty expression vector were used as a control. The cells were grown in synthetic glycerol-ethanol medium, and expression of the gene was induced after either 2 hours (early log phase) or 25 hours (stationary phase) by the addition of

galactose. The cells were then incubated for another 21 hours, after which they were harvested and assays were performed. We found that overexpression of PDAT had no significant effect on the growth rate as determined by the optical density. However, the total lipid content, measured as µmol fatty acids per mg yeast dry weight, was 47% (log phase) or 29% (stationary phase) higher in the PDAT overexpressing strain than in the control. Furthermore, the polar lipid and sterolester content was unaffected by overexpression of PDAT. Instead, the elevated lipid content in these cells is entirely due to an increased TAG content (Fig. 3A,B). Thus, the amount of TAG was increased by 2-fold in PDAT overexpressing early log phase cells and by 40% in stationary phase cells. It is interesting to note that a significant increase in the TAG content was achieved by overexpressing PDAT even under conditions (*i.e.* in stationary phase) where DAGAT is induced and thus contributes significantly to TAG synthesis. *In vitro* PDAT activity assayed in microsomes from the PDAT overexpressing strain was 7-fold higher than in the control strain, a finding which is consistent with the increased levels of TAG that we observed *in vivo* (Fig. 3C). These results clearly demonstrate the potential use of the PDAT gene in increasing the oil content in transgenic organisms.

Substrate specificity of yeast PDAT. The substrate specificity of yeast PDAT was analyzed using microsomes prepared from the PDAT overexpressing strain (see Fig. 4). The rate of TAG synthesis, under conditions given in figure 4 with di-oleoyl-PC as the acyl-donor, was 0.15 nmol per min and mg protein. With both oleoyl groups of PC labeled it was possible, under the given assay conditions, to detect the transfer of 11 pmol/min of [^{14}C]oleoyl chain into TAG and the formation of 15 pmol/min of lyso-PC. In microsomes from the PDAT-deficient strain, no TAG at all and only trace amounts of lyso-PC was detected, strongly suggesting that yeast PDAT catalyses the formation of equimolar amounts of TAG and lyso-PC when supplied with PC and DAG as substrates. The fact that somewhat more lyso-PC than TAG is formed can be

explained by the presence of a phospholipase in yeast microsomes, which produces lyso-PC and unesterified fatty acids from PC.

The specificity of yeast PDAT for different acyl group positions was investigated by incubating the microsomes with di-oleoyl-PC carrying a [^{14}C]acyl group either at the *sn*-1 position (Fig. 4A bar 2) or the *sn*-2 position (Fig. 4A bar 3). We found that the major ^{14}C-labeled product formed in the former case was lyso-PC, and in the latter case TAG. We conclude that yeast PDAT has a specificity for the transfer of acyl groups from the *sn*-2 position of the phospholipid to DAG, thus forming *sn*-1-lyso-PC and TAG. Under the given assay conditions, trace amounts of ^{14}C-labelled DAG is formed from the *sn*-1 labeled PC by the reversible action of a CDP-choline : choline phosphotransferase. This labeled DAG can then be further converted into TAG by the PDAT activity. It is therefore not possible to distinguish whether the minor amounts of labeled TAG that is formed in the presence of di-oleoyl-PC carrying a [^{14}C]acyl group in the *sn*-1 position, is synthesized directly from the *sn*-1-labeled PC by a PDAT that also can act on the *sn*-1 postion, or if it is first converted to *sn*-1-labeled DAG and then acylated by a PDAT with strict selectivity for the transfer of acyl groups at the *sn*-2 position of PC. Taken together, this shows that the PDAT encoded by YNR008w catalyses an acyl transfer from the *sn*-2 position of PC to DAG, thus causing the formation of TAG and lyso-PC.

The substrate specificity of yeast PDAT was further analyzed with respect to the headgroup of the acyl donor, the acyl group transferred and the acyl chains of the acceptor DAG molecule. The two major membrane lipids of *S. cerevisiae* are PC and PE, and as shown in Fig. 4B (bars 1 and 2), dioleoyl-PE is nearly 4-fold more efficient than dioleoyl-PC as acyl donor in the PDAT-catalyzed reaction. Moreover, the rate of acyl transfer is strongly dependent on the type of acyl group that is transferred. Thus, a ricinoleoyl group at the *sn*-2 position of PC is 2.5 times more efficiently transferred into TAG than an oleoyl

group in the same position (Fig. 4B bars 1 and 3). In contrast, yeast PDAT has no preference for the transfer of vernoloyl groups over oleoyl groups (Fig. 4B bars 1 and 4). The acyl chain of the acceptor DAG molecule also affects the efficiency of the reaction. Thus, DAG with a ricinoleoyl or a vernoloyl group is a more efficient acyl acceptor than dioleoyl-DAG (Fig. 4B bars 1, 5 and 6). Taken together, these results clearly show that the efficiency of the PDAT-catalyzed acyl transfer is strongly dependent on the properties of the substrate lipids.

PDAT genes. Nucleotide and amino acid sequences of several PDAT genes are given as SEQ ID No. 1 through 15. Futher provisional and/or partial sequences are given as SEQ ID NO 1a through 5a and 1b through 11b, respectively. One of the Arabidopsis genomic sequences (SEQ ID NO. 4) identfied an Arabidopsis EST cDNA clone; T04806. This cDNA clone was fully characterised and the nucleotide sequence is given as SEQ ID NO. 5. Based on the sequence homology of the T04806 cDNA and the *Arabidopsis thaliana* genomic DNA sequence (SEQ ID NO 4) it is apparent that an additional A is present at position 417 in the cDNA clone (data not shown). Excluding this nucleotide would give the amino acid sequence depicted in SEQ ID NO. 12.

Increased TAG content in seeds of Arabidopsis thaliana that express the yeast PDAT. For the expression of the yeast PDAT gene in *Arabidopsis thaliana* an EcoRI fragment from the pBluescript-PDAT was cloned together with napin promotor (25) into the vector pGPTV-KAN (26). A plasmid (pGNapPDAT) having the yeast PDAT gene in the correct orientation was identified and transformed into *Agrobacterium tumefaciens*. These bacteria were used to transform *Arabidopsis thaliana* columbia (C-24) plants using the root transformation method (27). Plants transformed with an empty vector were used as controls.

First generation seeds (T1) were harvested and germinated on kanamycin containing medium. Second generation seeds (T2) were pooled from individual plants and their fatty acid contents analysed by quantification of their methyl

esthers by gas liquid chromatography after methylation of the seeds with 2% sulphuric acid in methanol at 85 °C for 1,5 hours. Quantification was done with heptadecanoic acid methyl esters as internal standard.

From the transformation with pGNapPDAT one T1 plant (26-14) gave raise to seven T2 plants of which 3 plants yielded seeds with statistically (in a mean difference two-sided test) higher oil content than seeds from T2 plants generated from T1 plant 32-4 transformed with an empty vector (table 2).

References cited in the description:

1. Bell, R. M. & Coleman, R. A. (1980) *Annu. Rev. Biochem.* **49**, 459-487.
2. Stymne, S. & Stobart, K. (1987) in *The biochemistry of plants: a comprehensive treatsie, Vol. 9*, ed. Stumpf, P. K. (Academic Press, New York), pp. 175-214.
3. Cases, S. et al. (1998) *Proc. Natl. Acad. Sci. U S A* **95**, 13018-13023.
4. Hobbs, D. H., Lu, C. & Hills, M. J. (1999) *FEBS Lett.* **452**, 145-9
5. Zou, J., Wei, Y., Jako, C., Kumar, A., Selvaraj, G. & Taylor, D. C. (1999) *Plant J.* **19**, 645-653.
6. Lardizabal, K., Hawkins, D., Mai, J., & Wagner, N. (1999) Abstract presented at the Biochem. Mol. Plant Fatty Acids Glycerolipids Symposium, South Lake Tahoe, USA.
7. Thomas, B. J. & Rothstein, R. (1989) *Cell* **56**, 619-630.
8. Entian, K.-D. & Kötter, P. (1998) *Meth. Microbiol.* **26**, 431-449.
9. Kern, L., de Montigny, J., Jund, R. & Lacroute, F. (1990) *Gene* **88**, 149-157.
10. Ronne, H., Carlberg, M., Hu, G.-Z. & Nehlin, J. O. (1991) *Mol. Cell. Biol.* **11**, 4876-4884.
11. Stobart, K. & Stymne, S. (1990) in *Method in Plant Biochemistry, vol 4*, eds. Harwood, J. L. & Bowyer, J. R. (Academic press, London), pp. 19-46.
12. Bafor, M., Smith, M. A., Jonsson, L., Stobrt, A. K. & Stymne, S. (1991) *Biochem. J.* **280**, 507-514.
13. Banas, A., Johansson, I. & Stymne, S. (1992) *Plant Science* **84**, 137-144.
14. Kanda, P. & Wells, M. A. (1981) *J. Lipid. Res.* **22**, 877-879.
15. Ståhl, U., Ek, B. & Stymne, S. (1998) *Plant Physiol.* **117**, 197-205.
16. Stobart, K., Mancha, M. & Lenman M, Dahlqvist, A. & Stymne, S. (1997) *Planta* **203**, 58-66.
17. Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911-917.
18. Sherman, F., Fink, G. R. & Hicks, J. B. (1986) in *Laboratory Course Manual for Methods in Yeast Genentics* (Cold Spring Harbor Laboratory)
19. Meesters, P. A. E. P., Huijberts, G. N. M. and Eggink, G. (1996) *Appl. Microbiol. Biotechnol.* **45**, 575-579.
20. van de Loo, F. J., Fox, B. G. & Sommerville, C. (1993),in *Lipid metabolism in plants*, ed. Moore, T. S. (CRC Press, Inc.), pp. 91-126.
21. van de Loo, F. J., Broun, P., Turner, S. & Sommerville, S. (1995) *Proc. Natl.*

Acad. Sci. U S A **95**, 6743-6747.

22. Lee, M., Lenman, M., Banas, A., Bafor, M., Singh, S., Schweizer, M., Nilsson, R., Liljenberg, C., Dahlqvist, A., Gummeson, P-O., Sjödahl, S., Green, A., and Stymne, S. (1998) *Science* **280**, 915-918.
23. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997) *Nucl. Acids Res.* **24**, 4876-4882.
24. Saitou, N. & Nei, M. (1987) *Mol. Biol. Evol.* **4**, 406-425.
25. Stålberg, K., Ellerström, M., Josefsson, L., & Rask, L. (1993) *Plant Mol. Biol.* 23, 671
26. Becker, D., Kemper, E., Schell, J., Masterson, R. (1992) *Plant Mol. Biol.* 20, 1195
27. D. Valvekens, M. Van Montagu, and Van Lusbettens (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 5536

Description of Figures

FIG. 1.

Metabolism of ^{14}C-labeled PC into the neutral lipid fraction by plant microsomes. (A) Microsomes from developing seeds of sunflower, *R. communis* and *C. palaestina* were incubated for 80 min at 30°C with PC (8 nmol) having oleic acid in its *sn*-1 position, and either ^{14}C-labeled oleic, ricinoleic or vernolic acid in its *sn*-2 position. Radioactivity incorporated in TAG (open bars), DAG (solid bars), and unsterified fatty acids (hatched bars) was quantified using thin layer chromatography followed by electronic autoradiography, and is shown as percentage of added labeled substrate. (B) Synthesis *in vitro* of TAG carrying two vernoloyl and one [^{14}C]ricinoleoyl group by microsomes from *R. communis*. The substrates added were unlabeled divernoloyl-DAG (5 nmol), together with either *sn*-1-oleoyl-*sn*-2-[^{14}C]ricinoleoyl-DAG (0.4 nmol, 7700 dpm/nmol) or *sn*-1-oleoyl-*sn*-2-[^{14}C]ricinoleoyl-PC (0.4 nmol, 7700 dpm/nmol). The microsomes were incubated with the substrates for 30 min at 30°C, after which samples were removed for lipid analysis as described in the section „general methods". The data shown are the average of two experiments.

FIG. 2.

PDAT activity in yeast microsomes, as visualized by autoradiogram of neutral lipid products separated on TLC. Microsomal membranes (10 nmol of PC) from the wild type yeast strain FY1679 (lanes 1-3), a congenic yeast strain (FVKT004-04C(AL)) that is disrupted for YNR008w (lane 4) or the same disruption strain transformed with the plasmid pUS1, containing the YNR008w gene behind its native promotor (lane 5), were assayed for PDAT activity. As substrates, we used 2 nmol *sn*-1-oleoyl-*sn*-2-[^{14}C]ricinoleoyl-PC together with either 5 nmol of dioleoyl-DAG (lanes 2, 4 and 5) or *rac*-oleoyl-vernoleoyl-DAG (lane 3). The enzymatic assay and lipid analysis was performed as described in Materials and Methods. The cells were precultured for 20 h in liquid YPD

medium, harvested and re-suspended in an equal volume of minimal medium (19) containing 16 g/l glycerol. The cells were then grown for an additional 24 h prior to being harvested. Selection for the plasmid was maintained by growing the transformed cells in synthetic medium lacking uracil (18). Abbreviations: 1-OH-TAG, monoricinoleoyl-TAG; 1-OH-1-ep-TAG, monoricinoleoyl-monovernoloyl-TAG; OH-FA, unesterified ricinoleic acid.

Fig. 3.

Lipid content (A,B) and PDAT activity (C) in PDAT overexpressing yeast cells. The PDAT gene in the plasmid pUS4 was overexpressed from the galactose-induced *GAL1-TPK2* promotor in the wild type strain W303-1A (7). Its expression was induced after (A) 2 hours or (B) 25 hours of growth by the addition of 2% final concentration (w/v) of galactose. The cells were then incubated for another 22 hours before being harvested. The amount of lipids of the harvested cells was determined by GLC-analysis of its fatty acid contents and is presented as µmol fatty acids per mg dry weight in either TAG (open bar), polar lipids (hatched bar), sterol esters (solid bar) and other lipids (striped bar). The data shown are the mean values of results with three independent yeast cultures. (C) *In vitro* synthesis of TAG by microsomes prepared from yeast cells containing either the empty vector (vector) or the PDAT plasmid (+ PDAT). The cells were grown as in Fig. 3A. The substrate lipids dioleoyl-DAG (2.5 nmol) and *sn*-1-oleoyl-*sn*-2-[^{14}C]-oleoyl-PC (2 nmol) were added to aliquots of microsomes (10 nmol PC), which were then incubated for 10 min at 28 °C. The amount of label incorporated into TAG was quantified by electronic autoradiography. The results shown are the mean values of two experiments.

FIG. 4.

Substrate specificity of yeast PDAT. The PDAT activity was assayed by incubating aliquots of lyophilized microsomes (10 nmol PC) with substrate lipids at 30°C for 10 min (panel A) or 90 min (panel B). Unlabeled DAG (2.5 nmol) was used as substrates together with different labeled phospholipids, as shown

in the figure. (A) *Sn*-position specificity of yeast PDAT regarding the acyl donor substrate. Dioleoyl-DAG together with either *sn*-1-[^{14}C]oleoyl-*sn*-2-[^{14}C]oleoyl-PC (di-[^{14}C]-PC), *sn*-1-[^{14}C]oleoyl-*sn*-2-oleoyl-PC (*sn*1-[^{14}C]-PC) or *sn*-1-oleoyl-*sn*-2-[^{14}C]oleoyl-PC (*sn*2-[^{14}C]-PC). (B) Specificity of yeast PDAT regarding phospholipid headgroup and of the acyl composition of the phospholipid as well as of the diacylglycerol. Dioleoyl-DAG together with either *sn*-1-oleoyl-*sn*-2-[^{14}C]oleoyl-PC (oleoyl-PC), *sn*-1-oleoyl-*sn*-2-[^{14}C]oleoyl-PE (oleoyl-PE), *sn*-1-oleoyl-*sn*-2-[^{14}C]ricinoleoyl-PC (ricinoleoyl-PC) or *sn*-1-oleoyl-*sn*-2-[^{14}C]vernoloyl-PC (vernoloyl-PC). In the experiments presented in the 2 bars to the far right, monoricinoleoyl-DAG (ricinoleoyl-DAG or mono-vernoloyl-DAG (vernoloyl-DAG) were used together with *sn*-1-oleoyl-*sn*-2-[^{14}C]-oleoyl-PC. The label that was incorporated into TAG (solid bars) and lyso-PC (LPC, open bars) was quantified by electronic autoradiography. The results shown are the mean values of two experiments. The microsomes used were from W303-1A cells overexpressing the PDAT gene from the *GAL1-TPK2* promotor, as described in Fig. 3. The expression was induced at early stationary phase and the cells were harvested after an additional 24 h.

TAB.1:

In vitro synthesis of triacylglycerols in microsomal preparations of developing castor bean. Aliqouts of microsomes (20 nmol PC) were lyophilised and substrate lipids were added in benzene solution: (A) 0.4 nmol [^{14}C]-DAG (7760 dpm/nmol) and where indicated 1.6 nmol unlabelled DAG; (B) 0.4 nmol [^{14}C]-DAG (7760 dpm/nmol) and 5 nmol unlabelled di-ricinoleoyl-PC and (C) 0.25 nmol [^{14}C]-PC (4000 dpm/nmol) and 5 nmol unlabelled DAG. The benzene was evaporated by N_2 and 0.1 ml of 50 mM potassium phosphate was added, thoroughly mixed and incubated at 30 °C for (A) 20 min.; (B) and (C) 30 min.. Assays were terminated by extraction of the lipids in chloroform. The lipids were then separated by thin layer chromatography on silica gel 60 plates

(Merck; Darmstadt, Germany) in hexan/diethylether/acetic 35:70:1.5. The radioactive lipids were visualised and the radioactivity quantified on the plate by electronic autoradiography (Instant Imager, Packard, US). Results are presented as mean values of two experiments.

Radioactivity in different triacylglycerols (TAG) species formed. Abbreviations used: 1-OH-, mono-ricinoleoyl-; 2-OH, di-ricinoleoyl-; 3-OH-, triricinoleoyl; 1-OH-1-ver-, mono-ricinoleoly-monovernoleoyl-; 1-OH-2-ver-, mono-ricinoleoyl-divernoleoyl-. Radiolabelled DAG and PC were prepared enzymatically. The radiolabelled ricinoleoyl group is attached at the sn-2-position of the lipid and unlabelled oleoyl group at the sn-1-position. Unlabelled DAG with vernoleoyl- or ricinoleoyl chains were prepared by the action of TAG lipase (6) on oil of Euphorbia lagascae or Castor bean, respectively. Synthetic di-ricinoleoyl-PC was kindly provided from Metapontum Agribios (Italy).

TAB.2:

Total fatty acids per mg of T2 seeds pooled from individual *Arabidopsis thaliana* plants transformed with yeast PDAT gene under the control of napin promotor (26-14) or transformed with empty vector (32-4).

* = stastistical difference between control plants and PDAT transformed plants in a mean difference two-sided test at $\alpha = 5$.

Description of the SEQ ID:

SEQ ID NO. 1: Genomic DNA sequence and suggested amino acid sequence of the *Saccharomyces cerevisiae* PDAT gene, YNR008w, with GenBank accession number Z71623 and Y13139, and with nucleotide ID number 1302481.

SEQ ID NO. 2: The amino acid sequence of the suggested open reading frame YNR008w from *Saccharomyces cerevisiae.*

SEQ ID NO. 3: Genomic DNA sequence of the *Schizosaccharomyces pombe* gene SPBC776.14.

SEQ ID NO. 4: Genomic DNA sequence of part of the *Arabidopsis thaliana* locus with GenBank accession number AB006704.

SEQ ID NO. 5: Nucleotide sequence of the *Arabidopsis thaliana* cDNA clone with GenBank accession number T04806, and nucleotide ID number 315966.

SEQ ID NO. 6: Predicted amino acid sequence of the *Arabidopsis thaliana* cDNA clone with GenBank accession number T04806.

SEQ ID NO. 7: Nucleotide and amino acid sequence of the *Zea mays* EST clone with GenBank accession number AI491339, and nucleotide ID number 4388167.

SEQ ID NO. 8: Predicted amino acid sequence of the *Zea mays* EST clone with GenBank accession number AI491339, and nucleotide ID number 4388167.

SEQ ID NO. 9: DNA sequence of part of the *Neurospora crassa* EST clone W07G1, with GenBank accession number AI398644, and nucleotide ID number 4241729.

SEQ ID NO. 10: Genomic DNA sequence of part of the *Arabidopsis thaliana* locus with GenBank accession number AC004557.

SEQ ID NO. 11: Genomic DNA sequence of part of the *Arabidopsis thaliana* locus with GenBank accession number AC003027.

SEQ ID NO. 12: DNA sequencce of part of the *Lycopersicon esculentum* cDNA clone with GenBank accession number AI486635.

SEQ ID NO. 13: Amino acid sequence of the *Schizosaccharomyces pombe* putative open reading frame CAA22887 of the *Schizosaccharomyces pombe* gene SPBC776.14.

SEQ ID NO. 14: Amino acid sequence of the *Arabidopsis thaliana* putative open reading frame AAC80628 derived from the *Arabidopsis thaliana* locus with GenBank accession number AC004557.

SEQ ID NO 15: Amino acid sequence of the *Arabidopsis thaliana* putative open reading frame AAD10668 derived from the *Arabidopsis thaliana* locus with GenBank accession number AC003027.

Further provisional and/or partial sequences are defined through the following SEQ IDs:

SEQ ID NO. 1a: The amino acid sequence of the yeast ORF YNR008w from *Saccharomyces cerevisiae.*

SEQ ID NO. 2a: Amino acid sequence of the region of the *Arabidopsis thaliana* genomic sequence (AC004557).

SEQ ID NO. 3a: Amino acid sequence of the region of the *Arabidopsis thaliana* genomic sequence (AB006704).

SEQ ID NO. 4a: The corresponding genomic DNA sequence and amino acid sequence of the yeast ORF YNROO8w from *Saccharomyces cerevisiae.*

SEQ ID NO. 5a: The amino acid sequence of the yeast ORF YNROO8w from *Saccharomyces cerevisiae* derived form the corresponding genomic DNA sequence.

SEQ ID NO. 1b: Genomic DNA sequence of the *Saccharomyces cerevisiae* PDAT gene, YNR008w, genebank nucleotide ID number 1302481, and the suggested YNR008w amino acid sequence.

SEQ ID NO. 2b: The suggested amino acid sequence of the yeast gene YNR008w from *Saccharomyces cerevisiae.*

SEQ ID NO. 3b: Genomic DNA sequence of the *Schizosaccharomyces pombe* gene SPBC776.14.

SEQ ID NO. 4b: Genomic DNA sequence of part of the *Arabidopsis thaliana* locus with genebank accession number AB006704.

SEQ ID NO. 5b: Nucleotide sequence and the corresponding amino acid sequence of the *Arabidopsis thaliana* EST-clone with genebank accession number T04806, and ID number 315966.

SEQ ID NO. 6b: Nucleotide and amino acid sequence of the *Zea mays* cDNA clone with genebank ID number 4388167.

SEQ ID NO. 7b: Amino acid sequence of the *Zea mays* cDNA clone with genebank ID number 4388167.

SEQ ID NO. 8b: DNA sequence of part of the *Neurospora crassa* cDNA clone WO7G1, ID number 4241729.

SEQ ID NO. 9b: Genomic DNA sequence of part of the *Arabidopsis thaliana* locus with genebank accession number AC004557.

SEQ ID NO. 10b: Genomic DNA sequence of part of the *Arabidopsis thaliana* locus with genebank accession number AC003027.

SEQ ID NO. 11b: DNA sequence of part of the *Lycopersicon esculentum* cDNA clone with genebank accession number AI486635.

Claims

1. An enzyme catalysing in an acyl-CoA-independent reaction the transfer of fatty acids from phospholipids to diacylglycerol in the biosynthetic pathway for the production of triacylglycerol.

2. An enzyme according to claim 1, comprising an amino acid sequence as set forth in SEQ ID No. 2 or a functional fragment, derivate, allele, homolog or isoenzyme thereof.

3. An enzyme according to claims 1 or 2 designated as phospholipid:diacylglycerol acyltransferase (PDAT).

4. An enzyme according to claims 1 to 3, comprising an amino acid sequence as set forth in SEQ ID No. 1a, 2b or 5a or a functional fragment, derivate, allele, homolog or isoenzyme thereof.

5. An enzyme according to claims 1 to 4, comprising an amino acid sequence selected from the group consisting of sequences as set forth in SEQ ID No. 2a, 3a, 5b, 6, 7b, 8, 13, 14 or 15 or a functional fragment, derivate, allele, homolog or isoenzyme thereof.

6. An enzyme according to claims 1 to 5, comprising an amino acid sequence encoded through a nucleotide sequence, a portion, derivate, allele or homolog thereof selected from the group consisting of sequences as set forth in SEQ ID No. 1, 1b, 3, 3b, 4, 4a, 4b, 5, 5b, 6b, 7, 8b, 9, 9b, 10, 10b, 11, 11b or 12 or a functional fragment, derivate, allele, homolog or isoenzyme of the enzyme encoding amino acid sequence.

7. A nucleotide sequence encoding an enzyme catalysing in an acyl-CoA-independent reaction the transfer of fatty acids from phospholipids to

diacylglycerol in the biosynthetic pathway for the production of triacylglycerol.

8. A nucleotide sequence according to claim 7 encoding an enzyme designated as phospholipid:diacylglycerol acyltransferase (PDAT).

9. A nucleotide sequence according to claims 7 or 8, selected from the group consisting of sequences as set forth in SEQ ID No. 1, 1b, 3, 3b, 4, 4a, 4b, 9b, 10, 10b or 11 or a portion, derivate, allele or homolog thereof.

10. A partial nucleotide sequence corresponding to a fullength nucleotide sequence according to claims 7 to 9, selected from the group consisting of sequences as set forth in SEQ ID No. 5, 5b, 6b, 7, 8b, 9, 11b or 12 or a portion, derivate, allele or homolog thereof.

11. A nucleotide sequence according to claims 7 to 10, comprising a nucleotide sequence which is at least 40% homologous to a nucleotide sequence selected form the group consisting of those sequences set forth in SEQ ID No. 1, 1b, 3, 3b, 4, 4a, 4b, 5, 5b, 6b, 7, 8b, 9, 9b, 10, 10b, 11, 11b or 12.

12. A gene construct comprising a nucleotide sequence according to claims 7 to 11 operably linked to a heterologous nucleic acid.

13. A vector comprising a nucleotide sequence according to claims 7 to 11 or a gene construct according to claim 12.

14. A vector according to claim 13, which is an expression vector.

15. A vector according to claims 13 or 14, further comprising a selectable marker gene and/or nucleotide sequences for the replication in a host cell or the integration into the genome of the host cell.

16. A transgenic cell or organism containing a nucleotide sequence according to claims 7 to 11 and/or a gene construct according to claim 12 and/or a vector according to claims 13 to 15.

17. A transgenic cell or organism according to claim 16 which is an eucaryotic cell or organism.

18. A transgenic cell or organism according to claims 16 or 17 which is a yeast cell or a plant cell or a plant.

19. A transgenic cell or organism according to claims 16 to 18 having an altered biosynthetic pathway for the production of triacylglycerol.

20. A transgenic cell or organism according to claims 16 to 19 having an altered oil content.

21. A transgenic cell or organism according to claims 16 to 20 wherein the activity of PDAT is altered.

22. A transgenic cell or organism according to claims 16 to 21 wherein the altered activity of PDAT is characterized by an alteration in gene expression, catalytic activity and/or regulation of activity of the enzyme.

23. A transgenic cell or organism according to claims 16 to 22 wherein the altered biosynthetic pathway for the production of triacylglycerol is characterized by the prevention of accumulation of undesirable fatty acids in the membrane lipids.

24. A process for the production of triacylglycerol, comprising growing a transgenic cell or organism according to claims 16 to 23 under conditions

whereby the said nucleotide sequence according to claims 7 to 11 is expressed.

25. Triacylglycerols produced by a process according to claim 24.

26. Use of a nucleotide sequence according to claims 7 to 11 and/or an enzyme according to claims 1 to 6 for the production of triacylglycerol and/or triacylglycerols with uncommon fatty acids.

27. Use of a nucleotide sequence according to claims 7 to 11 and/or an enzyme according to claims 1 to 6 for the transformation of any cell or organism in order to be expressed in this cell or organism and result in an altered, preferably increased oil content of this cell or organism.

Figurs

Fig. 1:

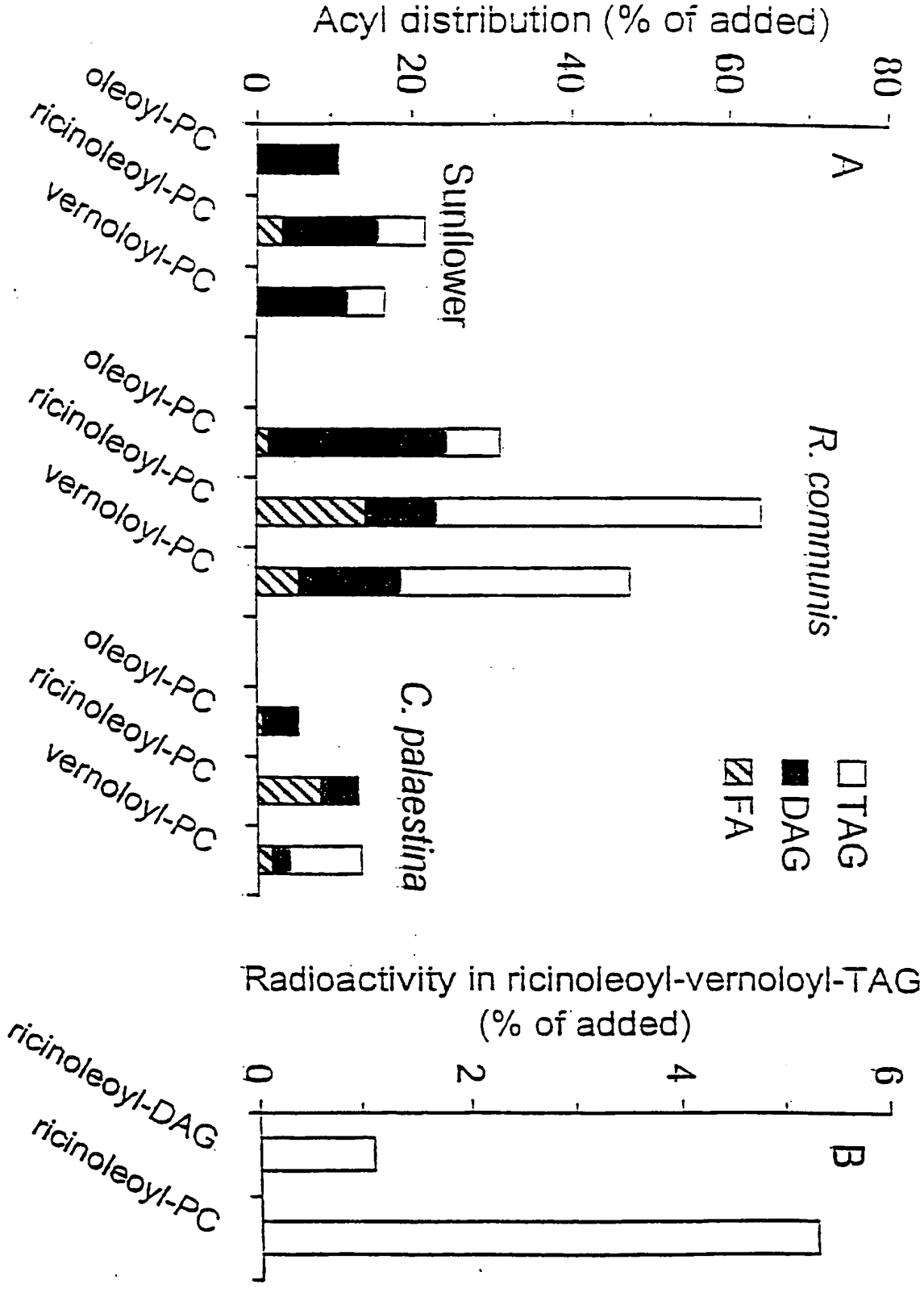
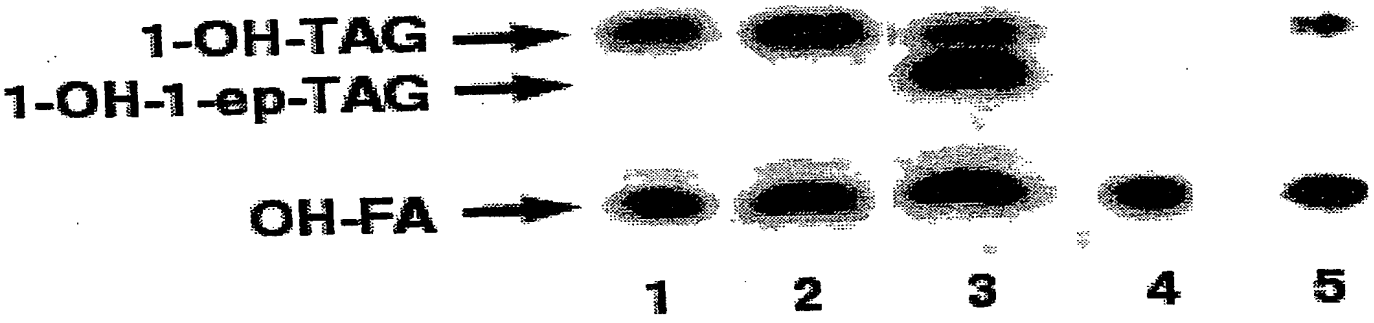
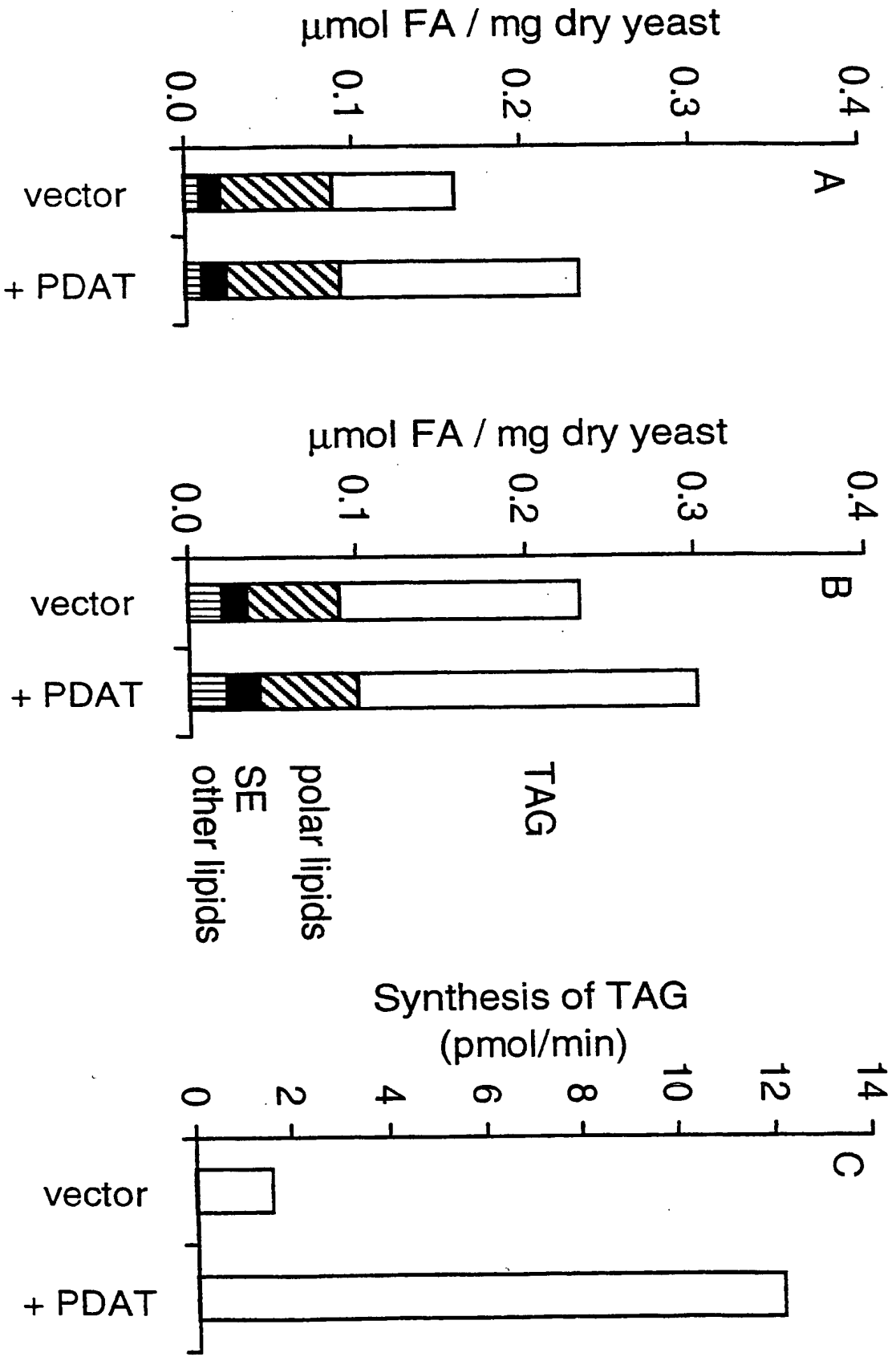
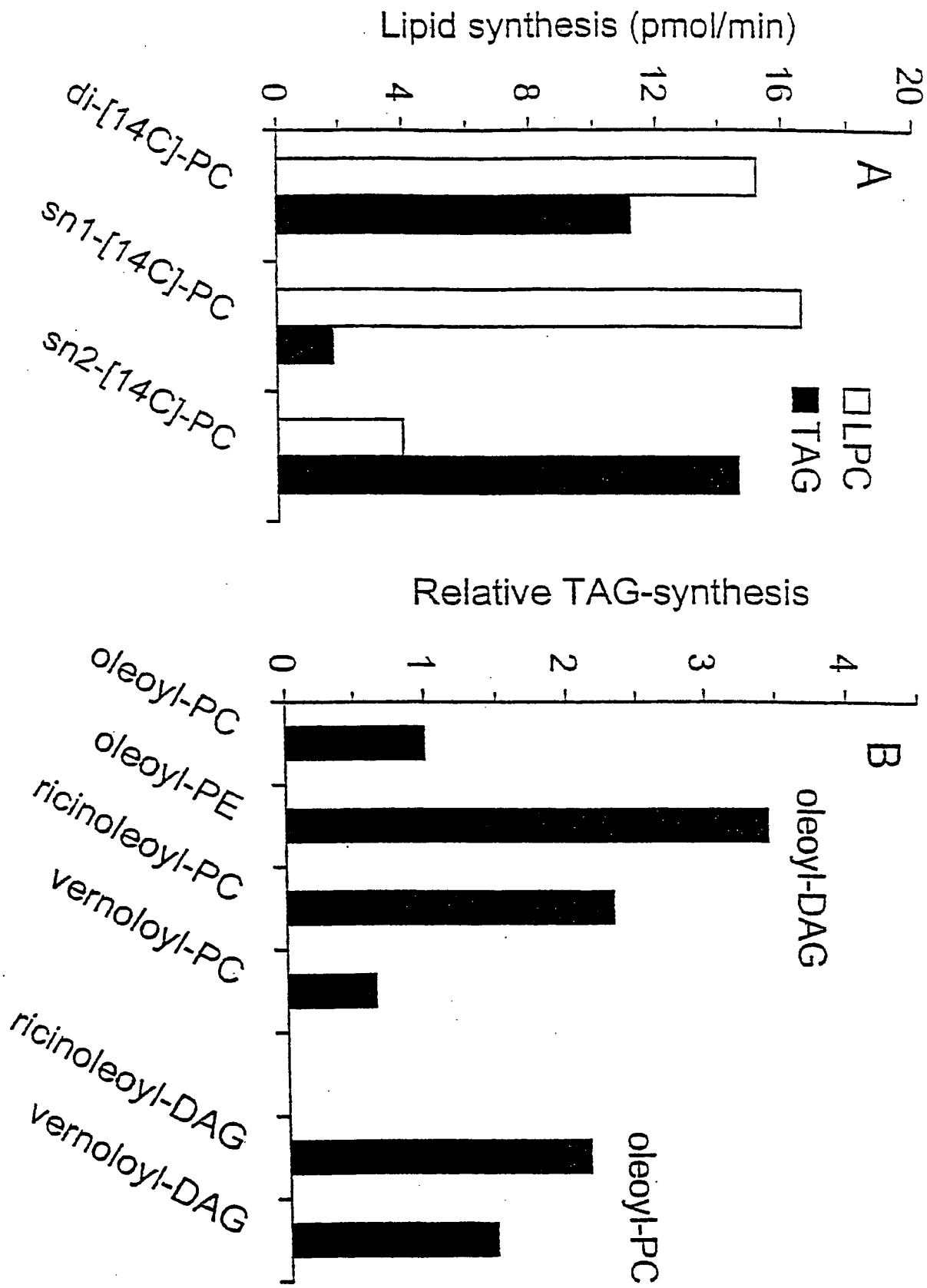


Fig 2







Tables

Tab. 1:

		mol % of added [^{14}C] -acyl group in TAG[1]				
Substrate added [^{14}C]-lipid[2]	**unlabelled lipid[2]**	**1-OH-TAG**	**2-OH-TAG**	**1-OH-1-ver-TAG**	**1-OH-2-ver-TAG**	**3-OH-TAG**
A mono-[^{14}C]-ricinoleoyl-DAG	mono-ricinoleoyl-DAG	2,8	12,4	-	-	-
A mono-[^{14}C]-ricinoleoyl-DAG	mono-vernoleoyl-DAG	3,2	12,1	1,3	-	-
A mono-[^{14}C]-ricinoleoyl-DAG	di-vernoleoyl-DAG	4	10	0,5	1,2	-
A mono-[^{14}C]-ricinoleoyl-DAG	di-ricinoleoyl-PC	0,3	24,8	-	-	-
B mono-[^{14}C]-ricinoleoyl-PC	none	6,8	8,0	-	-	4,7
C mono-[^{14}C]-ricinoleoyl-PC	di-oleoyl-DAG	8,6	9,8	-	-	5,0
C mono-[^{14}C]-ricinoleoyl-PC	mono-ricinoleoyl-DAG	5,7	16,7	-	-	1,9
C mono-[^{14}C]-ricinoleoyl-PC	di-ricinoleoyl-DAG	4,5	9,4	-	-	9,5
C mono-[^{14}C]-ricinoleoyl-PC	mono-vernoleoyl-DAG	6,0	11,5	10,9	0,5	7,4
C mono-[^{14}C]-ricinoleoyl-PC	di-vernoleoyl-DAG	6,7	10,8	1,1	8,4	6,8

Tab. 2:

T1 plant	T2 plant number	nmol fatty acids per mg seed	standard deviation
32-4	1	1277	±11 (n=2)
	4	1261	±63 (n=3)
	5	1369	±17 (n=3)
	6	1312	±53 (n=4)
	7	1197	±54 (n=5)
	8	1240	±78 (n=4)
	9	1283	±54 (n= 5)
	10	1381	±35 (n=5)
26-14	1	1444	±110 (n=4)
	2	1617*	±109 (n=4)
	3	1374	±37 (n=2)
	5	1562*	±70 (n=4)
	6	1393	±77 (n=4)
	7	1433	±98 (n=4)
	8	1581*	±82 (n=4)

Sequence Listing

<210> 1
<211> 1986
<212> genomic DNA
<213> Saccharomyces cerevisiae

<221> CDS
<222> (1)..(1983)

<400> 1

```
atg ggc aca ctg ttt cga aga aat gtc cag aac caa aag agt gat tct   48
Met Gly Thr Leu Phe Arg Arg Asn Val Gln Asn Gln Lys Ser Asp Ser
  1               5                  10                  15

gat gaa aac aat aaa ggg ggt tct gtt cat aac aag cga gag agc aga   96
Asp Glu Asn Asn Lys Gly Gly Ser Val His Asn Lys Arg Glu Ser Arg
             20                  25                  30

aac cac att cat cat caa cag gga tta ggc cat aag aga aga agg ggt   144
Asn His Ile His His Gln Gln Gly Leu Gly His Lys Arg Arg Arg Gly
         35                  40                  45

att agt ggc agt gca aaa aga aat gag cgt ggc aaa gat ttc gac agg   192
Ile Ser Gly Ser Ala Lys Arg Asn Glu Arg Gly Lys Asp Phe Asp Arg
     50                  55                  60

aaa aga gac ggg aac ggt aga aaa cgt tgg aga gat tcc aga aga ctg   240
Lys Arg Asp Gly Asn Gly Arg Lys Arg Trp Arg Asp Ser Arg Arg Leu
 65                  70                  75                  80

att ttc att ctt ggt gca ttc tta ggt gta ctt ttg ccg ttt agc ttt   288
Ile Phe Ile Leu Gly Ala Phe Leu Gly Val Leu Leu Pro Phe Ser Phe
                 85                  90                  95

ggc gct tat cat gtt cat aat agc gat agc gac ttg ttt gac aac ttt   336
Gly Ala Tyr His Val His Asn Ser Asp Ser Asp Leu Phe Asp Asn Phe
            100                 105                 110

gta aat ttt gat tca ctt aaa gtg tat ttg gat gat tgg aaa gat gtt   384
Val Asn Phe Asp Ser Leu Lys Val Tyr Leu Asp Asp Trp Lys Asp Val
        115                 120                 125

ctc cca caa ggt ata agt tcg ttt att gat gat att cag gct ggt aac   432
Leu Pro Gln Gly Ile Ser Ser Phe Ile Asp Asp Ile Gln Ala Gly Asn
    130                 135                 140
```

```
tac tcc aca tct tct tta gat gat ctc agt gaa aat ttt gcc gtt ggt   480
Tyr Ser Thr Ser Ser Leu Asp Asp Leu Ser Glu Asn Phe Ala Val Gly
145                 150                 155                 160

aaa caa ctc tta cgt gat tat aat atc gag gcc aaa cat cct gtt gta   528
Lys Gln Leu Leu Arg Asp Tyr Asn Ile Glu Ala Lys His Pro Val Val
                165                 170                 175

atg gtt cct ggt gtc att tct acg gga att gaa agc tgg gga gtt att   576
Met Val Pro Gly Val Ile Ser Thr Gly Ile Glu Ser Trp Gly Val Ile
            180                 185                 190

gga gac gat gag tgc gat agt tct gcg cat ttt cgt aaa cgg ctg tgg   624
Gly Asp Asp Glu Cys Asp Ser Ser Ala His Phe Arg Lys Arg Leu Trp
        195                 200                 205

gga agt ttt tac atg ctg aga aca atg gtt atg gat aaa gtt tgt tgg   672
Gly Ser Phe Tyr Met Leu Arg Thr Met Val Met Asp Lys Val Cys Trp
    210                 215                 220

ttg aaa cat gta atg tta gat cct gaa aca ggt ctg gac cca ccg aac   720
Leu Lys His Val Met Leu Asp Pro Glu Thr Gly Leu Asp Pro Pro Asn
225                 230                 235                 240

ttt acg cta cgt gca gca cag ggc ttc gaa tca act gat tat ttc atc   768
Phe Thr Leu Arg Ala Ala Gln Gly Phe Glu Ser Thr Asp Tyr Phe Ile
                245                 250                 255

gca ggg tat tgg att tgg aac aaa gtt ttc caa aat ctg gga gta att   816
Ala Gly Tyr Trp Ile Trp Asn Lys Val Phe Gln Asn Leu Gly Val Ile
            260                 265                 270

ggc tat gaa ccc aat aaa atg acg agt gct gcg tat gat tgg agg ctt   864
Gly Tyr Glu Pro Asn Lys Met Thr Ser Ala Ala Tyr Asp Trp Arg Leu
        275                 280                 285

gca tat tta gat cta gaa aga cgc gat agg tac ttt acg aag cta aag   912
Ala Tyr Leu Asp Leu Glu Arg Arg Asp Arg Tyr Phe Thr Lys Leu Lys
    290                 295                 300

gaa caa atc gaa ctg ttt cat caa ttg agt ggt gaa aaa gtt tgt tta   960
Glu Gln Ile Glu Leu Phe His Gln Leu Ser Gly Glu Lys Val Cys Leu
305                 310                 315                 320

att gga cat tct atg ggt tct cag att atc ttt tac ttt atg aaa tgg   1008
Ile Gly His Ser Met Gly Ser Gln Ile Ile Phe Tyr Phe Met Lys Trp
                325                 330                 335
```

```
gtc gag gct gaa ggc cct ctt tac ggt aat ggt ggt cgt ggc tgg gtt   1056
Val Glu Ala Glu Gly Pro Leu Tyr Gly Asn Gly Gly Arg Gly Trp Val
            340                 345                 350

aac gaa cac ata gat tca ttc att aat gca gca ggg acg ctt ctg ggc   1104
Asn Glu His Ile Asp Ser Phe Ile Asn Ala Ala Gly Thr Leu Leu Gly
        355                 360                 365

gct cca aag gca gtt cca gct cta att agt ggt gaa atg aaa gat acc   1152
Ala Pro Lys Ala Val Pro Ala Leu Ile Ser Gly Glu Met Lys Asp Thr
    370                 375                 380

att caa tta aat acg tta gcc atg tat ggt ttg gaa aag ttc ttc tca   1200
Ile Gln Leu Asn Thr Leu Ala Met Tyr Gly Leu Glu Lys Phe Phe Ser
385                 390                 395                 400

aga att gag aga gta aaa atg tta caa acg tgg ggt ggt ata cca tca   1248
Arg Ile Glu Arg Val Lys Met Leu Gln Thr Trp Gly Gly Ile Pro Ser
                405                 410                 415

atg cta cca aag gga gaa gag gtc att tgg ggg gat atg aag tca tct   1296
Met Leu Pro Lys Gly Glu Glu Val Ile Trp Gly Asp Met Lys Ser Ser
            420                 425                 430

tca gag gat gca ttg aat aac aac act gac aca tac ggc aat ttc att   1344
Ser Glu Asp Ala Leu Asn Asn Asn Thr Asp Thr Tyr Gly Asn Phe Ile
        435                 440                 445

cga ttt gaa agg aat acg agc gat gct ttc aac aaa aat ttg aca atg   1392
Arg Phe Glu Arg Asn Thr Ser Asp Ala Phe Asn Lys Asn Leu Thr Met
    450                 455                 460

aaa gac gcc att aac atg aca tta tcg ata tca cct gaa tgg ctc caa   1440
Lys Asp Ala Ile Asn Met Thr Leu Ser Ile Ser Pro Glu Trp Leu Gln
465                 470                 475                 480

aga aga gta cat gag cag tac tcg ttc ggc tat tcc aag aat gaa gaa   1488
Arg Arg Val His Glu Gln Tyr Ser Phe Gly Tyr Ser Lys Asn Glu Glu
                485                 490                 495

gag tta aga aaa aat gag cta cac cac aag cac tgg tcg aat cca atg   1536
Glu Leu Arg Lys Asn Glu Leu His His Lys His Trp Ser Asn Pro Met
            500                 505                 510

gaa gta cca ctt cca gaa gct ccc cac atg aaa atc tat tgt ata tac   1584
Glu Val Pro Leu Pro Glu Ala Pro His Met Lys Ile Tyr Cys Ile Tyr
        515                 520                 525
```

```
ggg gtg aac aac cca act gaa agg gca tat gta tat aag gaa gag gat   1632
Gly Val Asn Asn Pro Thr Glu Arg Ala Tyr Val Tyr Lys Glu Glu Asp
    530                 535                 540

gac tcc tct gct ctg aat ttg acc atc gac tac gaa agc aag caa cct   1680
Asp Ser Ser Ala Leu Asn Leu Thr Ile Asp Tyr Glu Ser Lys Gln Pro
545                 550                 555                 560

gta ttc ctc acc gag ggg gac gga acc gtt ccg ctc gtg gcg cat tca   1728
Val Phe Leu Thr Glu Gly Asp Gly Thr Val Pro Leu Val Ala His Ser
                565                 570                 575

atg tgt cac aaa tgg gcc cag ggt gct tca ccg tac aac cct gcc gga   1776
Met Cys His Lys Trp Ala Gln Gly Ala Ser Pro Tyr Asn Pro Ala Gly
            580                 585                 590

att aac gtt act att gtg gaa atg aaa cac cag cca gat cga ttt gat   1824
Ile Asn Val Thr Ile Val Glu Met Lys His Gln Pro Asp Arg Phe Asp
        595                 600                 605

ata cgt ggt gga gca aaa agc gcc gaa cac gta gac atc ctc ggc agc   1872
Ile Arg Gly Gly Ala Lys Ser Ala Glu His Val Asp Ile Leu Gly Ser
    610                 615                 620

gcg gag ttg aac gat tac atc ttg aaa att gca agc ggt aat ggc gat   1920
Ala Glu Leu Asn Asp Tyr Ile Leu Lys Ile Ala Ser Gly Asn Gly Asp
625                 630                 635                 640

ctc gtc gag cca cgc caa ttg tct aat ttg agc cag tgg gtt tct cag   1968
Leu Val Glu Pro Arg Gln Leu Ser Asn Leu Ser Gln Trp Val Ser Gln
                645                 650                 655

atg ccc ttc cca atg taa                                          1986
Met Pro Phe Pro Met
            660
```

<210> 2
<211> 661
<212> PRT
<213> Saccharomyces cerevisiae

<400> 2

```
Met Gly Thr Leu Phe Arg Arg Asn Val Gln Asn Gln Lys Ser Asp Ser
  1               5                  10                  15
Asp Glu Asn Asn Lys Gly Gly Ser Val His Asn Lys Arg Glu Ser Arg
             20                  25                  30
Asn His Ile His His Gln Gln Gly Leu Gly His Lys Arg Arg Arg Gly
         35                  40                  45
Ile Ser Gly Ser Ala Lys Arg Asn Glu Arg Gly Lys Asp Phe Asp Arg
     50                  55                  60
Lys Arg Asp Gly Asn Gly Arg Lys Arg Trp Arg Asp Ser Arg Arg Leu
 65                  70                  75                  80
Ile Phe Ile Leu Gly Ala Phe Leu Gly Val Leu Leu Pro Phe Ser Phe
                 85                  90                  95
Gly Ala Tyr His Val His Asn Ser Asp Ser Asp Leu Phe Asp Asn Phe
            100                 105                 110
Val Asn Phe Asp Ser Leu Lys Val Tyr Leu Asp Asp Trp Lys Asp Val
        115                 120                 125
Leu Pro Gln Gly Ile Ser Ser Phe Ile Asp Asp Ile Gln Ala Gly Asn
    130                 135                 140
Tyr Ser Thr Ser Ser Leu Asp Asp Leu Ser Glu Asn Phe Ala Val Gly
145                 150                 155                 160
Lys Gln Leu Leu Arg Asp Tyr Asn Ile Glu Ala Lys His Pro Val Val
                165                 170                 175
Met Val Pro Gly Val Ile Ser Thr Gly Ile Glu Ser Trp Gly Val Ile
            180                 185                 190
Gly Asp Asp Glu Cys Asp Ser Ser Ala His Phe Arg Lys Arg Leu Trp
        195                 200                 205
Gly Ser Phe Tyr Met Leu Arg Thr Met Val Met Asp Lys Val Cys Trp
    210                 215                 220
Leu Lys His Val Met Leu Asp Pro Glu Thr Gly Leu Asp Pro Pro Asn
225                 230                 235                 240
Phe Thr Leu Arg Ala Ala Gln Gly Phe Glu Ser Thr Asp Tyr Phe Ile
                245                 250                 255
Ala Gly Tyr Trp Ile Trp Asn Lys Val Phe Gln Asn Leu Gly Val Ile
            260                 265                 270
Gly Tyr Glu Pro Asn Lys Met Thr Ser Ala Ala Tyr Asp Trp Arg Leu
        275                 280                 285
Ala Tyr Leu Asp Leu Glu Arg Arg Asp Arg Tyr Phe Thr Lys Leu Lys
    290                 295                 300
Glu Gln Ile Glu Leu Phe His Gln Leu Ser Gly Glu Lys Val Cys Leu
305                 310                 315                 320
Ile Gly His Ser Met Gly Ser Gln Ile Ile Phe Tyr Phe Met Lys Trp
                325                 330                 335
```

Val Glu Ala Glu Gly Pro Leu Tyr Gly Asn Gly Gly Arg Gly Trp Val
340 345 350
Asn Glu His Ile Asp Ser Phe Ile Asn Ala Ala Gly Thr Leu Leu Gly
355 360 365
Ala Pro Lys Ala Val Pro Ala Leu Ile Ser Gly Glu Met Lys Asp Thr
370 375 380
Ile Gln Leu Asn Thr Leu Ala Met Tyr Gly Leu Glu Lys Phe Phe Ser
385 390 395 400
Arg Ile Glu Arg Val Lys Met Leu Gln Thr Trp Gly Gly Ile Pro Ser
405 410 415
Met Leu Pro Lys Gly Glu Glu Val Ile Trp Gly Asp Met Lys Ser Ser
420 425 430
Ser Glu Asp Ala Leu Asn Asn Asn Thr Asp Thr Tyr Gly Asn Phe Ile
435 440 445
Arg Phe Glu Arg Asn Thr Ser Asp Ala Phe Asn Lys Asn Leu Thr Met
450 455 460
Lys Asp Ala Ile Asn Met Thr Leu Ser Ile Ser Pro Glu Trp Leu Gln
465 470 475 480
Arg Arg Val His Glu Gln Tyr Ser Phe Gly Tyr Ser Lys Asn Glu Glu
485 490 495
Glu Leu Arg Lys Asn Glu Leu His His Lys His Trp Ser Asn Pro Met
500 505 510
Glu Val Pro Leu Pro Glu Ala Pro His Met Lys Ile Tyr Cys Ile Tyr
515 520 525
Gly Val Asn Asn Pro Thr Glu Arg Ala Tyr Val Tyr Lys Glu Glu Asp
530 535 540
Asp Ser Ser Ala Leu Asn Leu Thr Ile Asp Tyr Glu Ser Lys Gln Pro
545 550 555 560
Val Phe Leu Thr Glu Gly Asp Gly Thr Val Pro Leu Val Ala His Ser
565 570 575
Met Cys His Lys Trp Ala Gln Gly Ala Ser Pro Tyr Asn Pro Ala Gly
580 585 590
Ile Asn Val Thr Ile Val Glu Met Lys His Gln Pro Asp Arg Phe Asp
595 600 605
Ile Arg Gly Gly Ala Lys Ser Ala Glu His Val Asp Ile Leu Gly Ser
610 615 620
Ala Glu Leu Asn Asp Tyr Ile Leu Lys Ile Ala Ser Gly Asn Gly Asp
625 630 635 640
Leu Val Glu Pro Arg Gln Leu Ser Asn Leu Ser Gln Trp Val Ser Gln
645 650 655
Met Pro Phe Pro Met
660

<210> 3
<211> 2312
<212> genomic DNA
<213> Schizosaccharomyces pombe

<400> 3

```
ATGGCGTCTT CCAAGAAGAG CAAAACTCAT AAGAAAAAGA AAGAAGTCAA          50
ATCTCCTATC GACTTACCAA ATTCAAAGAA ACCAACTCGC GCTTTGAGTG         100
AGCAACCTTC AGCGTCCGAA ACACAATCTG TTTCAAATAA ATCAAGAAAA         150
TCTAAATTTG GAAAAAGATT GAATTTTATA TTGGGCGCTA TTTTGGGAAT         200
ATGCGGTGCT TTTTTTTTCG CTGTTGGAGA CGACAATGCT GTTTTCGACC         250
CTGCTACGTT AGATAAATTT GGGAATATGC TAGGCTCTTC AGACTTGTTT         300
GATGACATTA AAGGATATTT ATCTTATAAT GTGTTTAAGG ATGCACCTTT         350
TACTACGGAC AAGCCTTCGC AGTCTCCTAG CGGAAATGAA GTTCAAGTTG         400
GTCTTGATAT GTACAATGAG GGATATCGAA GTGACCATCC TGTTATTATG         450
GTTCCTGGTG TTATCAGCTC AGGATTAGAA AGTTGGTCGT TTAATAATTG         500
CTCGATTCCT TACTTTAGGA AACGTCTTTG GGGTAGCTGG TCTATGCTGA         550
AGGCAATGTT CCTTGACAAG CAATGCTGGC TTGAACATTT AATGCTTGAT         600
AAAAAAACCG GCTTGGATCC GAAGGGAATT AAGCTGCGAG CAGCTCAGGG         650
GTTTGAAGCA GCTGATTTTT TTATCACGGG CTATTGGATT TGGAGTAAAG         700
TAATTGAAAA CCTTGCTGCA ATTGGTTATG AGCCTAATAA CATGTTAAGT         750
GCTTCTTACG ATTGGCGGTT ATCATATGCA AATTTAGAGG AACGTGATAA         800
ATATTTTTCA AAGTTAAAAA TGTTCATTGA GTACAGCAAC ATTGTACATA         850
AGAAAAAGGT AGTGTTGATT TCTCACTCCA TGGGTTCACA GGTTACGTAC         900
TATTTTTTTA AGTGGGTTGA AGCTGAGGGC TACGGAAATG GTGGACCGAC         950
TTGGGTTAAT GATCATATTG AAGCATTTAT AAATGTGAGT CTCGATGGTT        1000
GTTTGACTAC GTTTCTAACT TTTGAATAGA TATCGGGATC TTTGATTGGA        1050
GCACCCAAAA CAGTGGCAGC GCTTTTATCG GGTGAAATGA AAGATACAGG        1100
TATTGTAATT ACATTAAACA TGTTAATATT TAATTTTTGC TAACCGTTTT        1150
AAGCTCAATT GAATCAGTTT TCGGTCTATG GGTAAGCAAT AAATTGTTGA        1200
GATTTGTTAC TAATTTACTG TTTAGTTTGG AAAAATTTTT TTCCCGTTCT        1250
GAGGTATATT CAAAAATACA AATGTGCTCT ACTTTTTCTA ACTTTTAATA        1300
GAGAGCCATG ATGGTTCGCA CTATGGGAGG AGTTAGTTCT ATGCTTCCTA        1350
AAGGAGGCGA TGTTGTATGG GGAAATGCCA GTTGGGTAAG AAATATGTGC        1400
TGTTAATTTT TTATTAATAT TTAGGCTCCA GATGATCTTA ATCAAACAAA        1450
TTTTTCCAAT GGTGCAATTA TTCGATATAG AGAAGACATT GATAAGGACC        1500
ACGATGAATT TGACATAGAT GATGCATTAC AATTTTTAAA AAATGTTACA        1550
GATGACGATT TTAAAGTCAT GCTAGCGAAA AATTATTCCC ACGGTCTTGC        1600
TTGGACTGAA AAAGAAGTGT TAAAAAATAA CGAAATGCCG TCTAAATGGA        1650
TAAATCCGCT AGAAGTAAGA ACATTAAAGT TACTAAATTA TACTAACCCA        1700
AATAGACTAG TCTTCCTTAT GCTCCTGATA TGAAAATTTA TTGCGTTCAC        1750
GGGGTCGGAA AACCAACTGA GAGAGGTTAT TATTATACTA ATAATCCTGA        1800
GGGGCAACCT GTCATTGATT CCTCGGTTAA TGATGGAACA AAAGTTGAAA        1850
ATGTGAGAGA ATTTATGTTT CAAACATTCT ATTAACTGTT TTATTAGGGT        1900
ATTGTTATGG ATGATGGTGA TGGAACTTTA CCAATATTAG CCCTTGGTTT        1950
GGTGTGCAAT AAAGTTTGGC AAACAAAAAG GTTTAATCCT GCTAATACAA        2000
GTATCACAAA TTATGAAATC AAGCATGAAC CTGCTGCGTT TGATCTGAGA        2050
GGAGGACCTC GCTCGGCAGA ACACGTCGAT ATACTTGGAC ATTCAGAGCT        2100
AAATGTATGT TCATTTTACC TTACAAATTT CTATTACTAA CTCTTGAAAT        2150
AAGGAAATTA TTTTAAAAGT TTCATCAGGC CATGGTGACT CGGTACCAAA        2200
CCGTTATATA TCAGATATCC AGTACGGACA TAAGTTTTGT AGATTGCAAT        2250
TAACTAACTA ACCGAACAGG GAAATAATAA ATGAGATAAA TCTCGATAAA        2300
CCTAGAAATT AA                                                2312
```

<210> 4
<211> 3685
<212> genomic DNA
<213> Arabidopsis thaliana

<400> 4

```
ATGCCCCTTA TTCATCGGAA AAAGCCGACG GAGAAACCAT CGACGCCGCC          50
ATCTGAAGAG GTGGTGCACG ATGAGGATTC GCAAAAGAAA CCACACGAAT         100
CTTCCAAATC CCACCATAAG AAATCGAACG GAGGAGGGAA GTGGTCGTGC         150
ATCGATTCTT GTTGTTGGTT CATTGGGTGT GTGTGTGTAA CCTGGTGGTT         200
TCTTCTCTTC CTTTACAACG CAATGCCTGC GAGCTTCCCT CAGTATGTAA         250
CGGAGCGAAT CACGGGTCCT TTGCCTGACC CGCCCGGTGT TAAGCTCAAA         300
AAAGAAGGTC TTAAGGCGAA ACATCCTGTT GTCTTCATTC CTGGGATTGT         350
CACCGGTGGG CTCGAGCTTT GGGAAGGCAA ACAATGCGCT GATGGTTTAT         400
TTAGAAAACG TTTGTGGGGT GGAACTTTTG GTGAAGTCTA CAAAAGGTGA         450
GCTCAACAAT TCTCACTCTT CCTTTATATT GGGATTTGGA TTGGATCTGA         500
TGAGATCACG CACTTGTTGC TTCTTCAACA TCACTCAAAC TTTAATTCCA         550
TGTTTGTCTG TCTTACTCTT TACTTTTTTT TTTTTTGAT GTGAAACGCT         600
ATTTTCTTAA GAGACTATTT CTGTATGTGT AAGGTAAGCG TTCCAAGGAC         650
GTAATTGGCT TGGACTATTT CTGTTTGATT GTTAACTTTA GGATATAAAA         700
TAGCTGCCTT GGAATTTCAA GTCATCTTAT TGCCAAATCT GTTGCTAGAC         750
ATGCCCTAGA GTCCGTTCAT AACAAGTTAC TTCCTTTACT GTCGTTGCGT         800
GTAGATTTAG CTTTGTGTAG CGTATAATGA AGTAGTGTTT TATGTTTTGT         850
TGGGAATAGA GAAGTTCTAA CTACATCTGT GGAAAGTGTG TTCAGGCTGT         900
GATAGAGGAC TGTTGCTTTA TTATTCAACT ATGTATATGT GTAATTAAAG         950
CTAGTTCCTT TTTGATCTTT CAGCTCAATG TGCTTTTCTC AATTTTTTTC    1000
TCAATTTCAA AGTTTCACAT CGAGTTTATT CACATGTCTT GAATTTCGTC         1050
CATCCTCGTT CTGTTATCCA GCTTTGAACT CCTCCCGACC CTGCTATGGA         1100
TATATTAAAA AAAAAGTGTT TTGTGGGTTG CATCTTTGTT ACGATCTGCA         1150
TCTTCTTCTT TCGGCTCAGT GTTCATGTTT TTGCTATGGT AGAGATGGGC         1200
AATGTTATTG TTGATGGTAA CAGTGGTATA GTTGATAGTA TCTTAACTAA         1250
TCAATTATCT CTTTGATTCA GGCCTCTATG TTGGGTGGAA CACATGTCAC    1300
TTGACAATGA AACTGGGTTG GATCCAGCTG GTATTAGAGT TCGAGCTGTA    1350
TCAGGACTCG TGGCTGCTGA CTACTTTGCT CCTGGCTACT TTGTCTGGGC    1400
AGTGCTGATT GCTAACCTTG CACATATTGG ATATGAAGAG AAAAATATGT    1450
ACATGGCTGC ATATGACTGG CGGCTTTCGT TTCAGAACAC AGAGGTTCTT    1500
TTCTCATCGT TCTTTCTATT ATTCTGTTCC ATGTTACGTT TCTTTCTTCA    1550
TTACTTAAGG CTTAAATATG TTTCATGTTG AATTAATAGG TACGTGATCA         1600
GACTCTTAGC CGTATGAAAA GTAATATAGA GTTGATGGTT TCTACCAACG    1650
GTGGAAAAAA AGCAGTTATA GTTCCGCATT CCATGGGGGT CTTGTATTTT    1700
CTACATTTTA TGAAGTGGGT TGAGGCACCA GCTCCTCTGG GTGGCGGGGG    1750
TGGGCCAGAT TGGTGTGCAA AGTATATTAA GGCGGTGATG AACATTGGTG    1800
GACCATTTCT TGGTGTTCCA AAAGCTGTTG CAGGGCTTTT CTCTGCTGAA    1850
GCAAAGGATG TTGCAGTTGC CAGGTATTGA ATATCTGCTT ATACTTTTGA    1900
TGATCAGAAC CTTGGCTCTG GAACTCAAAG TTATTCTACT AAATATCAAT    1950
TCTAATAACA TTGCTATATT ATCGCTGCAA CTGACATTGG TTGATTATTT    2000
TTGCTGCTTA TGTAACTGAA ACTCTCTTGA GATTAGACAA ATGATGAATT    2050
GATAATTCTT ACGCATTGCT CTGTGATGAC CAGTTTCTTA GCTTCGACGA    2100
TAACATTTGT CATACTGTCT TTTGGAGGGC ATTGAATTTT GCTATGGAAA         2150
GCGCTGGAGC TTCCATGCTT GCATTCTTTA CCAATTAGCG TTATTCTGCT         2200
TCTTTCAATT TTCTTGTATA TGCATCTATG GTCTTTTATT TCTTCTTAAT         2250
TAAAGACTCG TTGGATTAGT TGCTCTATTA GTCACTTGGT TCCTTAATAT         2300
AGAACTTTAC TTTCTTCGAA AATTGCAGAG CGATTGCCCC AGGATTCTTA         2350
GACACCGATA TATTTAGACT TCAGACCTTG CAGCATGTAA TGAGAATGAC         2400
ACGCACATGG GACTCAACAA TGTCTATGTT ACCGAAGGGA GGTGACACGA         2450
TATGGGGCGG GCTTGATTGG TCACCGGAGA AAGGCCACAC CTGTTGTGGG         2500
AAAAAGCAAA AGAACAACGA AACTTGTGGT GAAGCAGGTG AAAACGGAGT         2550
TTCCAAGAAA AGTCCTGTTA ACTATGGAAG GATGATATCT TTTGGGAAAG         2600
AAGTAGCAGA GGCTGCGCCA TCTGAGATTA ATAATATTGA TTTTCGAGTA         2650
AGGACATATA AATCATAATA AACCTTGTAC ATTTTGTGAT TGTATGATGA         2700
ATATCTGTAC ATTTTATCTG GTGAAGGGTG CTGTCAAAGG TCAGAGTATC         2750
CCAAATCACA CCTGTCGTGA CGTGTGGACA GAGTACCATG ACATGGGAAT         2800
TGCTGGGATC AAAGCTATCG CTGAGTATAA GGTCTACACT GCTGGTGAAG         2850
```

```
CTATAGATCT ACTACATTAT GTTGCTCCTA AGATGATGGC GCGTGGTGCC          2900
GCTCATTTCT CTTATGGAAT TGCTGATGAT TTGGATGACA CCAAGTATCA          2950
AGATCCCAAA TACTGGTCAA ATCCGTTAGA GACAAAGTAA GTGATTTCTT          3000
GATTCCAACT GTATCCTTCG TCCTGATGCA TTATCAGTCT TTTTGTTTTC          3050
GGTCTTGTTG GATATGGTTT TCAGCTCAAA GCTTACAAAG CTGTTTCTGA          3100
GCCTTTCTCA AAAAGGCTTG CTCAGTAATA TTGAGGTGCT AAAGTTGATA          3150
CATGTGACTC TTGCTTATAA ATCCTCCGTT TGGTTTGTTC TGCTTTTTCA          3200
GATTACCGAA TGCTCCTGAG ATGGAAATCT ACTCATTATA CGGAGTGGGG          3250
ATACCAACGG AACGAGCATA CGTATACAAG CTTAACCAGT CTCCCGACAG     3300
TTGCATCCCC TTTCAGATAT TCACTTCTGC TCACGAGGAG GACGAAGATA          3350
GCTGTCTGAA AGCAGGAGTT TACAATGTGG ATGGGGATGA AACAGTACCC          3400
GTCCTAAGTG CCGGGTACAT GTGTGCAAAA GCGTGGCGTG GCAAGACAAG          3450
ATTCAACCCT TCCGGAATCA AGACTTATAT AAGAGAATAC AATCACTCTC          3500
CGCCGGCTAA CCTGTTGGAA GGGCGCGGGA CGCAGAGTGG TGCCCATGTT          3550
GATATCATGG GAAACTTTGC TTTGATCGAA GATATCATGA GGGTTGCCGC          3600
CGGAGGTAAC GGGTCTGATA TAGGACATGA CCAGGTCCAC TCTGGCATAT          3650
TTGAATGGTC GGAGCGTATT GACCTGAAGC TGTGA                3685
```

<210> 5
<211> 2427
<212> cDNA
<213> Arabidopsis thaliana

<400> 5

```
AGAAACAGCT CTTTGTCTCT CTCGACTGAT CTAACAATCC CTAATCTGTG          50
TTCTAAATTC CTGGACGAGA TTTGACAAAG TCCGTATAGC TTAACCTGGT         100
TTAATTTCAA GTGACAGATA TGCCCCTTAT TCATCGGAAA AAGCCGACGG         150
AGAAACCATC GACGCCGCCA TCTGAAGAGG TGGTGCACGA TGAGGATTCG         200
CAAAAGAAAC CACACGAATC TTCCAAATCC CACCATAAGA AATCGAACGG         250
AGGAGGGAAG TGGTCGTGCA TCGATTCTTG TTGTTGGTTC ATTGGGTGTG         300
TGTGTGTAAC CTGGTGGTTT CTTCTCTTCC TTTACAACGC AATGCCTGCG         350
AGCTTCCCTC AGTATGTAAC GGAGCGAATC ACGGGTCCTT TGCCTGACCC         400
GCCCGGTGTT AAGCTCAAAA AAAGAAGGTC TTAAGGCGAA ACATCCTGTT    450
GTCTTCATTC CTGGGATTGT CACCGGTGGG CTCGAGCTTT GGGAAGGCAA         500
ACAATGCGCT GATGGTTTAT TTAGAAAACG TTTGTGGGGT GGAACTTTTG         550
GTGAAGTCTA CAAAAGGCCT CTATGTTGGG TGGAACACAT GTCACTTGAC         600
AATGAAACTG GGTTGGATCC AGCTGGTATT AGAGTTCGAG CTGTATCAGG         650
ACTCGTGGCT GCTGACTACT TTGCTCCTGG CTACTTTGTC TGGGCAGTGC         700
TGATTGCTAA CCTTGCACAT ATTGGATATG AAGAGAAAAA TATGTACATG         750
GCTGCATATG ACTGGCGGCT TTCGTTTCAG AACACAGAGG TACGTGATCA         800
GACTCTTAGC CGTATGAAAA GTAATATAGA GTTGATGGTT TCTACCAACG         850
GTGGAAAAAA AGCAGTTATA GTTCCGCATT CCATGGGGGT CTTGTATTTT         900
CTACATTTTA TGAAGTGGGT TGAGGCACCA GCTCCTCTGG GTGGCGGGGG         950
TGGGCCAGAT TGGTGTGCAA AGTATATTAA GGCGGTGATG AACATTGGTG    1000
GACCATTTCT TGGTGTTCCA AAAGCTGTTG CAGGGCTTTT CTCTGCTGAA        1050
GCAAAGGATG TTGCAGTTGC CAGAGCGATT GCCCCAGGAT TCTTAGACAC        1100
CGATATATTT AGACTTCAGA CCTTGCAGCA TGTAATGAGA ATGACACGCA        1150
CATGGGACTC AACAATGTCT ATGTTACCGA AGGGAGGTGA CACGATATGG        1200
GGCGGGCTTG ATTGGTCACC GGAGAAAGGC CACACCTGTT GTGGGAAAAA        1250
GCAAAAGAAC AACGAAACTT GTGGTGAAGC AGGTGAAAAC GGAGTTTCCA        1300
AGAAAAGTCC TGTTAACTAT GGAAGGATGA TATCTTTTGG GAAAGAAGTA        1350
GCAGAGGCTG CGCCATCTGA GATTAATAAT ATTGATTTTC GAGGTGCTGT        1400
CAAAGGTCAG AGTATCCCAA ATCACACCTG TCGTGACGTG TGGACAGAGT        1450
ACCATGACAT GGGAATTGCT GGGATCAAAG CTATCGCTGA GTATAAGGTC        1500
TACACTGCTG GTGAAGCTAT AGATCTACTA CATTATGTTG CTCCTAAGAT        1550
GATGGCGCGT GGTGCCGCTC ATTTCTCTTA TGGAATTGCT GATGATTTGG        1600
ATGACACCAA GTATCAAGAT CCCAAATACT GGTCAAATCC GTTAGAGACA        1650
AAATTACCGA ATGCTCCTGA GATGGAAATC TACTCATTAT ACGGAGTGGG        1700
GATACCAACG GAACGAGCAT ACGTATACAA GCTTAACCAG TCTCCCGACA        1750
GTTGCATCCC CTTTCAGATA TTCACTTCTG CTCACGAGGA GGACGAAGAT        1800
AGCTGTCTGA AAGCAGGAGT TTACAATGTG GATGGGGATG AAACAGTACC        1850
CGTCCTAAGT GCCGGGTACA TGTGTGCAAA AGCGTGGCGT GGCAAGACAA        1900
GATTCAACCC TTCCGGAATC AAGACTTATA TAAGAGAATA CAATCACTCT        1950
CCGCCGGCTA ACCTGTTGGA AGGGCGCGGG ACGCAGAGTG GTGCCCATGT        2000
TGATATCATG GGAAACTTTG CTTTGATCGA AGATATCATG AGGGTTGCCG        2050
CCGGAGGTAA CGGGTCTGAT ATAGGACATG ACCAGGTCCA CTCTGGCATA        2100
TTTGAATGGT CGGAGCGTAT TGACCTGAAG CTGTGAATAT CATGATCTCT        2150
TTAAGCTGTC CTGTCAGCTT ATGTGAATCC AATACTTTGA AAGAGAGATC        2200
ATCATCAATT CATCATCATC GTCATCATCA TGATGCTCAA CTCACAAAGA        2250
AGCCTGAGAA TGATACTTTG GTgCGAAATT CTCAATACCT CTTTAATATT        2300
CTTATTGAAT GTAAATTATA CAATCCTATC TAATGTTTGA ACGATAACAC        2350
AAAACTTGCT GCNGCCATGT TTGTTTGTCT TGTCAAAAGC ATCAATTTGT        2400
GGGTTAAAAA AAAAAAAAAA AAAAAAA                  2427
```

<210> 6
<211> 671
<212> PRT
<213> Arabidopsis thaliana

<400> 6

```
MPLIHRKKPT EKPSTPPSEE VVHDEDSQKK PHESSKSHHK KSNGGGKWSC          50
IDSCCWFIGC VCVTWWFLLF LYNAMPASFP QYVTERITGP LPDPPGVKLK         100
KEGLKAKHPV VFIPGIVTGG LELWEGKQCA DGLFRKRLWG GTFGEVYKRP         150
LCWVEHMSLD NETGLDPAGI RVRAVSGLVA ADYFAPGYFV WAVLIANLAH         200
IGYEEKNMYM AAYDWRLSFQ NTEVRDQTLS RMKSNIELMV STNGGKKAVI         250
VPHSMGVLYF LHFMKWVEAP APLGGGGGPD WCAKYIKAVM NIGGPFLGVP         300
KAVAGLFSAE AKDVAVARAI APGFLDTDIF RLQTLQHVMR MTRTWDSTMS         350
MLPKGGDTIW GGLDWSPEKG HTCCGKKQKN NETCGEAGEN GVSKKSPVNY         400
GRMISFGKEV AEAAPSEINN IDFRGAVKGQ SIPNHTCRDV WTEYHDMGIA         450
GIKAIAEYKV YTAGEAIDLL HYVAPKMMAR GAAHFSYGIA DDLDDTKYQD         500
PKYWSNPLET KLPNAPEMEI YSLYGVGIPT ERAYVYKLNQ SPDSCIPFQI         550
FTSAHEEDED SCLKAGVYNV DGDETVPVLS AGYMCAKAWR GKTRFNPSGI         600
KTYIREYNHS PPANLLEGRG TQSGAHVDIM GNFALIEDIM RVAAGGNGSD         650
IGHDQVHSGI FEWSERIDLK L                    671
```

<210> 7
<211> 643
<212> cDNA
<213> Zea mays

<221> CDS
<222> (1)..(402)

<400> 6

```
CGG GAG AAA ATA GCT GCT TTG AAG GGG GGT GTT TAC TTA GCC GAT GGT 48
Arg Glu Lys Ile Ala Ala Leu Lys Gly Gly Val Tyr Leu Ala Asp Gly
  1               5                  10                  15

GAT GAA ACT GTT CCA GTT CTT AGT GCG GGC TAC ATG TGT GCG AAA GGA 96
Asp Glu Thr Val Pro Val Leu Ser Ala Gly Tyr Met Cys Ala Lys Gly
            20                  25                  30

TGG CGT GGC AAA ACT CGT TTC AGC CCT GCC GGC AGC AAG ACT TAC GTG 144
Trp Arg Gly Lys Thr Arg Phe Ser Pro Ala Gly Ser Lys Thr Tyr Val
        35                  40                  45

AGA GAA TAC AGC CAT TCG CCA CCC TCT ACT CTC CTG GAA GGC AGG GGC 192
Arg Glu Tyr Ser His Ser Pro Pro Ser Thr Leu Leu Glu Gly Arg Gly
    50                  55                  60

ACC CAG AGC GGT GCA CAT GTT GAT ATA ATG GGG AAC TTT GCT CTA ATT 240
Thr Gln Ser Gly Ala His Val Asp Ile Met Gly Asn Phe Ala Leu Ile
65                  70                  75                  80

GAG GAC GTC ATC AGA ATA GCT GCT GGG GCA ACC GGT GAG GAA ATT GGT 288
Glu Asp Val Ile Arg Ile Ala Ala Gly Ala Thr Gly Glu Glu Ile Gly
                85                  90                  95

GGC GAT CAG GTT TAT TCA GAT ATA TTC AAG TGG TCA GAG AAA ATC AAA 336
Gly Asp Gln Val Tyr Ser Asp Ile Phe Lys Trp Ser Glu Lys Ile Lys
            100                 105                 110

TTG AAA TTG TAA  CCTATGGGAA GTTAAAGAAG TGCCGACCCG TTTATTGCGTTCC 391
Leu Lys Leu
        115

AAAGTGTCCT GCCTGAGTGC AACTCTGGAT TTTGCTTAAA TATTGTAATT TTTCACGC 449
TTCATTCGTC CCTTTGTCAA ATTTACATTT GACAGGACGC CAATGCGATA CGATGTTG 507
TACCGCTATT TTCAGCATTG TATATTAAAC TGTACAGGTG TAAGTTGCAT TTGCCAGC 565
TGAAATTGTG TAGTCGTTTT CTTTACGATT TAATANCAAG TGGCGGAGCA GTGCCCCA 623
AGCNAAAAAA AAAAAAAAAA                643
```

<210> 8
<211> 115
<212> PRT
<213> Zea mays

<400> 8

```
Arg Glu Lys Ile Ala Ala Leu Lys Gly Gly Val Tyr Leu Ala Asp Gly
  1               5                  10                  15

Asp Glu Thr Val Pro Val Leu Ser Ala Gly Tyr Met Cys Ala Lys Gly
             20                  25                  30

Trp Arg Gly Lys Thr Arg Phe Ser Pro Ala Gly Ser Lys Thr Tyr Val
         35                  40                  45

Arg Glu Tyr Ser His Ser Pro Pro Ser Thr Leu Leu Glu Gly Arg Gly
     50                  55                  60

Thr Gln Ser Gly Ala His Val Asp Ile Met Gly Asn Phe Ala Leu Ile
65                  70                  75                  80

Glu Asp Val Ile Arg Ile Ala Ala Gly Ala Thr Gly Glu Glu Ile Gly
                85                  90                  95

Gly Asp Gln Val Tyr Ser Asp Ile Phe Lys Trp Ser Glu Lys Ile Lys
            100                 105                 110

Leu Lys Leu
        115
```

<210> 9
<211> 616
<212> cDNA
<213> Neurospora crassa

<400> 9
ggtggcgaag acganggcgg aagttggagg ctaacgagaa tgacnctcgg 50
agatggatct accctctaga gacacgacta ccnttgcacc cagcctcaag 100
gtntacngtt tntatgggta ggaagccgac ggagcgagcc tacatctatc 150
tggcgcccga tcccgggacg acaacgcatc tttagatgac gatcgatacg 200
actttgactn aggggcacat tgaccacggt gtgattttgg gcgaaggcga 250
tggcacagtg aaccttatga gtttggggta cctgtgcaat aaggggtgga 300
aaatgaagag atacaatcct gcgggctcaa aaataaccgt ggtcgagatg 350
ccgcatgaac cagaacggtt caatccgaga ggagggccga atacggcgga 400
tcacgtggat attctaggaa ggcagaatct aaacgagtac attcttaaag 450
tggcggcagg tcgaggcgat acaattgagg attttattac tagtaatatt 500
cttaaatatg tagaaaaggt tgaaatttat gaagagtaat taaatacggc 550
acataggtta ctcaatagta tgactaatta aaaaaaaatt ttttttctaa 600
aaaaaaaaaa aaaaaa 616

<210> 10
<211> 1562
<212> genomic DNA
<213> Arabidopsis thaliana

<400> 10
ATGAAAAAAA TATCTTCACA TTATTCGGTA GTCATAGCGA TACTCGTTGT 50
GGTGACGATG ACCTCGATGT GTCAAGCTGT GGGTAGCAAC GTGTACCCTT 100
TGATTCTGGT TCCAGGAAAC GGAGGTAACC AGCTAGAGGT ACGGCTGGAC 150
AGAGAATACA AGCCAAGTAG TGTCTGGTGT AGCAGCTGGT TATATCCGAT 200
TCATAAGAAG AGTGGTGGAT GGTTTAGGCT ATGGTTCGAT GCAGCAGTGT 250
TATTGTCTCC CTTCACCAGG TGCTTCAGCG ATCGAATGAT GTTGTACTAT 300
GACCCTGATT TGGATGATTA CCAAAATGCT CCTGGTGTCC AAACCCGGGT 350
TCCTCATTTC GGTTCGACCA AATCACTTCT ATACCTCGAC CCTCGTCTCC 400
GGTTAGTACT TTCCAAGATA TATCATTTTG GGACATTTGC ATAATGAACA 450
AAATAGACAT AAATTTGGGG GATTATTGTT ATATCAATAT CCATTTATAT 500
GCTAGTCGGT AATGTGAGTG TTATGTTAGT ATAGTTAATG TGAGTGTTAT 550
GTGATTTTCC ATTTTAAATG AAGCTAGAAA GTTGTCGTTT AATAATGTTG 600
CTATGTCATG AGAATTATAA GGACACTATG TAAATGTAGC TTAATAATAA 650
GGTTTGATTT GCAGAGATGC CACATCTTAC ATGGAACATT TGGTGAAAGC 700
TCTAGAGAAA AAATGCGGGT ATGTTAACGA CCAAACCATC CTAGGAGCTC 750
CATATGATTT CAGGTACGGC CTGGCTGCTT CGGGCCACCC GTCCCGTGTA 800
GCCTCACAGT TCCTACAAGA CCTCAAACAA TTGGTGGAAA AAACTAGCAG 850
CGAGAACGAA GGAAAGCCAG TGATACTCCT CTCCCATAGC CTAGGAGGAC 900
TTTTCGTCCT CCATTTCCTC AACCGTACCA CCCCTTCATG GCGCCGCAAG 950
TACATCAAAC ACTTTGTTGC ACTCGCTGCG CCATGGGGTG GGACGATCTC 1000
TCAGATGAAG ACATTTGCTT CTGGCAACAC ACTCGGTGTC CCTTTAGTTA 1050
ACCCTTTGCT GGTCAGACGG CATCAGAGGA CCTCCGAGAG TAACCAATGG 1100
CTACTTCCAT CTACCAAAGT GTTTCACGAC AGAACTAAAC CGCTTGTCGT 1150
AACTCCCCAG GTTAACTACA CAGCTTACGA GATGGATCGG TTTTTTGCAG 1200
ACATTGGATT CTCACAAGGA GTTGTGCCTT ACAAGACAAG AGTGTTGCCT 1250
TTAACAGAGG AGCTGATGAC TCCGGGAGTG CCAGTCACTT GCATATATGG 1300
GAGAGGAGTT GATACACCGG AGGTTTTGAT GTATGGAAAA GGAGGATTCG 1350
ATAAGCAACC AGAGATTAAG TATGGAGATG GAGATGGGAC GGTTAATTTG 1400
GCGAGCTTAG CAGCTTTGAA AGTCGATAGC TTGAACACCG TAGAGATTGA 1450
TGGAGTTTCG CATACATCTA TACTTAAAGA CGAGATCGCA CTTAAAGAGA 1500
TTATGAAGCA GATTTCAATT ATTAATTATG AATTAGCCAA TGTTAATGCC 1550
GTCAATGAAT GA 1562

<210> 11
<211> 3896
<212> genomic DNA
<213> Arabidopsis thaliana

<400> 11

ATGGGAGCGA ATTCGAAATC AGTAACGGCT TCCTTCACCG TCATCGCCGT 50
TTTTTTCTTG ATTTGCGGTG GCCGAACTGC GGTGGAGGAT GAGACCGAGT 100
TTCACGGCGA CTACTCGAAG CTATCGGGTA TAATCATTCC GGGATTTGCG 150
TCGACGCAGC TACGAGCGTG GTCGATCCTT GACTGTCCAT ACACTCCGTT 200
GGACTTCAAT CCGCTCGACC TCGTATGGCT AGACACCACT AAGGTCCGTG 250
ATCTTCATTT CCTTCGCTCC TTATTCTGTC GGTCGAGTCA CTTGTTGATG 300
AATTCCAAGC GAAATATAGC AATGAAGCAT GTCTCGTCTC TCTTATTGAT 350
TCGTTCATTA GTCAACAGTG ACGCTTCTGA ATCTGAGTTT AGAGTCATAT 400
AAAACAGCTG ACTCGGCGAG TGTTTCCCAT CGCTTTTGGT TCGCTAAATG 450
TAGCGCAATG AATGTGTAAT TAGTCTGCGC TTTTTATTCA ACTAGATCTG 500
CAAGTTTTTC AGAGTGCTCA ATAGTAGTTA GAAAATGTTA GGTCATTTTA 550
CTTGTGCATT GTGATTCTTT TGGTTGTTGC TTACTGATCG ACGTGATGGA 600
TGGTTTACAG CTTCTTTCTG CTGTCAACTG CTGGTTTAAG TGTATGGTGC 650
TAGATCCTTA TAATCAAACA GACCATCCCG AGTGTAAGTC ACGGCCTGAC 700
AGTGGTCTTT CAGCCATCAC AGAATTGGAT CCAGGTTACA TAACAGGTAG 750
TTTCGGATTT TTCTTTCTTT TGAGTTTTCT TCAATTTGAT ATCATCTTGT 800
TGTGATATAA TATGGCTAAG TTCATTAATT TGGTCAATTT TCAGGTCCTC 850
TTTCTACTGT CTGGAAAGAG TGGCTTAAGT GGTGTGTTGA GTTTGGTATA 900
GAAGCAAATG CAATTGTCGC TGTTCCATAC GATTGGAGAT TGTCACCAAC 950
CAAATTGGAA GAGCGTGACC TTTACTTTCA CAAGCTCAAG TTAGTCCTTA 1000
TCAGGCTAAT GTCTTTTATC TTCTCTTTTT ATGTAAGATA AGCTAAGAGC 1050
TCTGGTCGTC TTCCTTTTTG CAGGTTGACC TTTGAAACTG CTTTAAAACT 1100
CCGTGGCGGC CCTTCTATAG TATTTGCCCA TTCAATGGGT AATAATGTCT 1150
TCAGATACTT TCTGGAATGG CTGAGGCTAG AAATTGCACC AAAACATTAT 1200
TTGAAGTGGC TTGATCAGCA TATCCATGCT TATTTCGCTG TTGGTACCGG 1250
CCTACTATCC TTAAGTTACC ATTTTATTTT TTCTCTAATT GGGGGAGTTA 1300
TGTTGTGACT TACTGGATTG AGCTCGATAC CTGATTTGTT GTTGATTTAG 1350
GAGCTCCTCT TCTTGGTTCT GTTGAGGCAA TCAAATCTAC TCTCTCTGGT 1400
GTAACGTTTG GCCTTCCTGT TTCTGAGGTG ACCTCTGACT TCTCTTTAGT 1450
TTTAAGTAGT TGATATCAAC CAGGTCTTAT AACTCACTGG ATTTTCCTTT 1500
TGAAAGTATT ACTTTTGTTA ATTGAACTGC TGTACGCGAT ATGGTATCTG 1550
TAGATCTTGA AGTGCTAGTT ATCAAAGAAC ATATTGTGGG TAGTATACCT 1600
GTCAGCGGCC TTAGCTAATA CAACCAAACC ACATGTACAC TGATTTAGTT 1650
TTCAGATTAT TATGGTAGAC TTTAAGTTGA GAAGAAACTT TGACTGAAAT 1700
CTTTTTATTT TAATAGGCTA TGATTTGTTT ATTGAAATCA TGTGACATAT 1750
TGACATGCGC TTCTCATGTT TTTTGTTGGC AAGGCTTCAG GGAACTGCTC 1800
GGTTGTTGTC CAATTCTTTT GCGTCGTCAT TGTGGCTTAT GCCATTTTCA 1850
AAGAATTGCA AGGGTGATAA CACATTCTGG ACGCATTTTT CTGGGGGTGC 1900
TGCAAAGAAA GATAAGCGCG TATACCACTG TGATGAAGAG GAATATCAAT 1950
CAAAATATTC TGGCTGGCCG ACAAATATTA TTAACATTGA AATTCCTTCC 2000
ACTAGCGGTT AGACTCTGTA TATGCAACTG TAACACTAAC AAAAGTTTCA 2050
CCAAGAATGT TCACTCTCAT ATTTCGTTCC TTTGATGTGT ATCCATCAGT 2100
TACAGAAACA GCTCTAGTCA ACATGACCAG CATGGAATGT GGCCTTCCCA 2150
CCCTTTTGTC TTTCACAGCC CGTGAACTAG CAGATGGGAC TCTTTTCAAA 2200
GCAATAGAAG ACTATGACCC AGATAGCAAG AGGATGTTAC ACCAGTTAAA 2250
GAAGTACGTA CCTTTCTTTG TGATAAGAAA TATTGCTCAT CGATCATCAC 2300
TTGCTGGCTT CTTGTACGTC AAATTGTTTT GTTTAAATCT CTATATCAAT 2350
TGTTCATATG CTTTGTCTTT CTTACTATAA GAAACAAGTA TAATCAGAAA 2400
CCTTATTATT GATTATCAGT TCTCTCCTTA TATTATGGAA TGTCTTTTTC 2450
GTTTACAGTT ATGAATGCAA AAGGGGGTAT TTTAGTTGAT TGATTCTCTC 2500
ATTCTCTAGT TTGTTTTGAC TAATAGCGTC AATTTTGTTT TTCTAGCAAA 2550
TCTTTGTGAA TTATATATAA CATGCTAACT ATACTTTTCA GGTTGTATCA 2600
TGATGACCCT GTTTTTAATC CTCTGACTCC TTGGGAGAGA CCACCTATAA 2650
AAAATGTATT TTGCATATAT GGTGCTCATC TAAAGACAGA GGTATGATGC 2700
ATTCTCAATA TCACATTATG CGTTGACTTT GTTATTATAT TCCCCATTTG 2750
GTTTGCAATA TCTTTTTGAA TTATGATTTA TCTTCTCCCT TGCATCTTAT 2800
GCTATTAAGC GTTAAAGGTA CTAAATGTAT GAAGCTGTCT GTCATAGGTT 2850

```
GGTTATTACT TTGCCCCAAG TGGCAAACCT TATCCTGATA ATTGGATCAT          2900
CACGGATATC ATTTATGAAA CTGAAGGTTC CCTCGTGTCA AGGTAATTTT          2950
CCGCAATGGC AGAAGTAAAA CAGGAAGGCA AAGTCTTCTG TATCAGTCTA          3000
GTGGCATGTT ATCTCAGTTG CATAAGCAAA TTATTAAACA ACTAAAATTT          3050
AAGTACTTTT TTATCATTCC TTTTGAGCTT AGTGGATGAT CAGTGGCTTA          3100
AAGTGGGAAG AGGTGTTGCA TGAAACATGA CACTTGTATC AAAGATAACT          3150
AGCAAAACAA AACTAACCCA TTTCTGAATT TCATATTATT AGGAGTAGTC          3200
GTGCTTTTAA AAAATTTGTT TTAAGAAACC GAAAAACTAG TTCATATCTT          3250
GATTGTGCAA TATCTGCAGG TCTGGAACTG TGGTTGATGG GAACGCTGGA  3300
CCTATAACTG GGGATGAGAC GGTAAGCTCA GAAGTTGGTT TTGAAATTAT          3350
CTTCTTGCAA ACTACTGAAG ACTAAGATAA TACTTGCTTC TGGAACACTG          3400
CTTGCTATGT TCTCTAGTAC ACTGCAATAT TGACTCTCCG CTACTTTTAT          3450
TGATTATGAA ATTGATCTCT TATAGGTACC CTATCATTCA CTCTCTTGGT          3500
GCAAGAATTG GCTCGGACCT AAAGTTAACA TAACAATGGC TCCCCAGGTA          3550
CTCTTTTTTA GTTCCTCACC TTATATAGAT CAAACTTTAA GTGTACTTTT          3600
CTGGTTATGT GTTGATTTAC CTCCAATTTG TTCTTTCTAA AAATCATATA          3650
TCTCTGTACT CCTCAAGAAC TTGTATTAAT CTAAACGAGA TTCTCATTGG          3700
GAAAATAAAA CAACAGCCAG AACACGATGG AAGCGACGTA CATGTGGAAC          3750
TAAATGTTGA TCATGAGCAT GGGTCAGACA TCATAGCTAA CATGACAAAA          3800
GCACCAAGGG TTAAGTACAT AACCTTTTAT GAAGACTCTG AGAGCATTCC          3850
GGGGAAGAGA ACCGCAGTCT GGGAGCTTGA TAAAAGTGGG TATTAA      3896
```

<210> 12
<211> 709
<212> cDNA
<213> Lycopersicon esculentum

<400> 12

```
CTGGGGCCAA AAGTGAACAT AACAAGGACA CCACAGTCAG AGCATGATGT          50
TCAGATGTAC AAGTGCATCT AAATATAGAG CATCAACATG GTGAAGATAT         100
CATTCCCAAT ATGACAAAGT TACCTACAAT GAAGTACATA ACCTATTATG         150
AGGATTCTGA AAGTTTTCCA GGGACAAGAA CAGCAGTTTG GGAGCTTGAT         200
AAAGCAAATC ACAGGAACAT TGTCAGATCT CCAGCTTTGA TGCGGGAGCT         250
GTGGCTTGAG ATGTGGCATG ATATTCATCC TGATAAAAAG TCCAAGTTTG         300
TTACAAAAGG TGGTGTCTGA TCCTCACTAT TTTCTTCTAT AAATGTTTGA         350
GTTTGTATTG ACATTGTAAG TATTGCAACA AAAAGCAAAG CGTGGGCCTC         400
TGAGGGATGA GGACTGCTAT TGGGATTACG GGAAAGCTCG ATGTGCATGG         450
GCTGAACATT GTGAATACAG GTTAGAATAT TCAAATTATA TTTTGCAAAA         500
TATTCTCTTT TTGTGTATTT AGGCCACCTT TCCCCGGTCA CAACGATGCA         550
GATATGTATT CGGGGATGTT CACCTGGGAC AGAGTTGCAG ATTGAAGAGT         600
TCTACATCTC ACATCCTGTC ACACTATGTG TGATATTTAA GAAACTTTGT         650
TTGGCGGAAC AACAAGTTTG CACAAACATT TGAAGAAGAA AGCGAAATGA         700
TTCAGAGAG                                                     709
```

<210> 13
<211> 623
<212> PRT
<213> *Schizosaccharomyces pombe*

<400> 13

MASSKKSKTHKKKKEVKSPIDLPNSKKPTRALSEQPSASETQSVSNKSRKSKFGKRLNFILGAILGICGA70
FFFAVGDDNAVFDPATLDKFGNMLGSSDLFDDIKGYLSYNVFKDAPFTTDKPSQSPSGNEVQVGLDMYNE140
GYRSDHPVIMVPGVISSGLESWSFNNCSIPYFRKRLWGSWSMLKAMFLDKQCWLEHLMLDKKTGLDPKGI210
KLRAAQGFEAADFFITGYWIWSKVIENLAAIGYEPNNMLSASYDWRLSYANLEERDKYFSKLKMFIEYSN280
IVHKKKVVLISHSMGSQVTYYFFKWVEAEGYGNGGPTWVNDHIEAFINISGSLIGAPKTVAALLSGEMKD350
TGIVITLNILEKFFSRSERAMMVRTMGGVSSMLPKGGDVAPDDLNQTNFSNGAIIRYREDIDKDHDEFDI420
DDALQFLKNVTDDDFKVMLAKNYSHGLAWTEKEVLKNNEMPSKWINPLETSLPYAPDMKIYCVHGVGKPT490
ERGYYYTNNPEGQPVIDSSVNDGTKVENGIVMDDGDGTLPILALGLVCNKVWQTKRFNPANTSITNYEIK560
HEPAAFDLRGGPRSAEHVDILGHSELNEIILKVSSGHGDSVPNRYISDIQEIINEINLDKPRN 623

<210> 14
<211> 432
<212> PRT
<213> *Arabidopsis thaliana*

<400> 14
MKKISSHYSVVIAILVVVTMTSMCQAVGSNVYPLILVPGNGGNQLEVRLDREYKPSSVWCSSWLYPIHKK70
SGGWFRLWFDAAVLLSPFTRCFSDRMMLYYDPDLDDYQNAPGVQTRVPHFGSTKSLLYLDPRLRDATSYM140
EHLVKALEKKCGYVNDQTILGAPYDFRYGLAASGHPSRVASQFLQDLKQLVEKTSSENEGKPVILLSHSL210
GGLFVLHFLNRTTPSWRRKYIKHFVALAAPWGGTISQMKTFASGNTLGVPLVNPLLVRRHQRTSESNQWL280
LPSTKVFHDRTKPLVVTPQVNYTAYEMDRFFADIGFSQGVVPYKTRVLPLTEELMTPGVPVTCIYGRGVD350
TPEVLMYGKGGFDKQPEIKYGDGDGTVNLASLAALKVDSLNTVEIDGVSHTSILKDEIALKEIMKQISII420
NYELANVNAVNE 432

<210> 15
<211> 552
<212> PRT
<213> *Arabidopsis thaliana*

<400> 15

MGANSKSVTASFTVIAVFFLICGGRTAVEDETEFHGDYSKLSGIIIPGFASTQLRAWSILDCPYTPLDFN70
PLDLVWLDTTKLLSAVNCWFKCMVLDPYNQTDHPECKSRPDSGLSAITELDPGYITGPLSTVWKEWLKWC140
VEFGIEANAIVAVPYDWRLSPTKLEERDLYFHKLKLTFETALKLRGGPSIVFAHSMGNNVFRYFLEWLRL210
EIAPKHYLKWLDQHIHAYFAVGAPLLGSVEAIKSTLSGVTFGLPVSEGTARLLSNSFASSLWLMPFSKNC280
KGDNTFWTHFSGGAAKKDKRVYHCDEEEYQSKYSGWPTNIINIEIPSTSARELADGTLFKAIEDYDPDSK350
RMLHQLKKYVPFFVIRNIAHRSSLAGFLLYHDDPVFNPLTPWERPPIKNVFCIYGAHLKTEVGYYFAPSG420
KPYPDNWIITDIIYETEGSLVSRSGTVVDGNAGPITGDETVPYHSLSWCKNWLGPKVNITMAPQILIGKI490
KQQPEHDGSDVHVELNVDHEHGSDIIANMTKAPRVKYITFYEDSESIPGKRTAVWELDKSGY 552

<170> PatentIn Ver. 2.0

<210> 10
<211> 661
<212> PRT
<213> Saccharomyces cerevisiae

<400> 1

```
Met Gly Thr Leu Phe Arg Arg Asn Val Gln Asn Gln Lys Ser Asp Ser
 1               5                  10                  15

Asp Glu Asn Asn Lys Gly Gly Ser Val His Asn Lys Arg Glu Ser Arg
            20                  25                  30

Asn His Ile His His Gln Gln Gly Leu Gly His Lys Arg Arg Arg Gly
        35                  40                  45

Ile Ser Gly Ser Ala Lys Arg Asn Glu Arg Gly Lys Asp Phe Asp Arg
    50                  55                  60

Lys Arg Asp Gly Asn Gly Arg Lys Arg Trp Arg Asp Ser Arg Arg Leu
65                  70                  75                  80

Ile Phe Ile Leu Gly Ala Phe Leu Gly Val Leu Leu Pro Phe Ser Phe
                85                  90                  95

Gly Ala Tyr His Val His Asn Ser Asp Ser Asp Leu Phe Asp Asn Phe
            100                 105                 110

Val Asn Phe Asp Ser Leu Lys Val Tyr Leu Asp Asp Trp Lys Asp Val
        115                 120                 125

Leu Pro Gln Gly Ile Ser Ser Phe Ile Asp Asp Ile Gln Ala Gly Asn
    130                 135                 140

Tyr Ser Thr Ser Ser Leu Asp Asp Leu Ser Glu Asn Phe Ala Val Gly
145                 150                 155                 160

Lys Gln Leu Leu Arg Asp Tyr Asn Ile Glu Ala Lys His Pro Val Val
                165                 170                 175

Met Val Pro Gly Val Ile Ser Thr Gly Ile Glu Ser Trp Gly Val Ile
            180                 185                 190

Gly Asp Asp Glu Cys Asp Ser Ser Ala His Phe Arg Lys Arg Leu Trp
        195                 200                 205
```

Gly Ser Phe Tyr Met Leu Arg Thr Met Val Met Asp Lys Val Cys Trp
210 215 220

Leu Lys His Val Met Leu Asp Pro Glu Thr Gly Leu Asp Pro Pro Asn
225 230 235 240

Phe Thr Leu Arg Ala Ala Gln Gly Phe Glu Ser Thr Asp Tyr Phe Ile
245 250 255

Ala Gly Tyr Trp Ile Trp Asn Lys Val Phe Gln Asn Leu Gly Val Ile
260 265 270

Gly Tyr Glu Pro Asn Lys Met Thr Ser Ala Ala Tyr Asp Trp Arg Leu
275 280 285

Ala Tyr Leu Asp Leu Glu Arg Arg Asp Arg Tyr Phe Thr Lys Leu Lys
290 295 300

Glu Gln Ile Glu Leu Phe His Gln Leu Ser Gly Glu Lys Val Cys Leu
305 310 315 320

Ile Gly His Ser Met Gly Ser Gln Ile Ile Phe Tyr Phe Met Lys Trp
325 330 335

Val Glu Ala Glu Gly Pro Leu Tyr Gly Asn Gly Gly Arg Gly Trp Val
340 345 350

Asn Glu His Ile Asp Ser Phe Ile Asn Ala Ala Gly Thr Leu Leu Gly
355 360 365

Ala Pro Lys Ala Val Pro Ala Leu Ile Ser Gly Glu Met Lys Asp Thr
370 375 380

Ile Gln Leu Asn Thr Leu Ala Met Tyr Gly Leu Glu Lys Phe Phe Ser
385 390 395 400

Arg Ile Glu Arg Val Lys Met Leu Gln Thr Trp Gly Gly Ile Pro Ser
405 410 415

Met Leu Pro Lys Gly Glu Glu Val Ile Trp Gly Asp Met Lys Ser Ser
420 425 430

Ser Glu Asp Ala Leu Asn Asn Asn Thr Asp Thr Tyr Gly Asn Phe Ile
435 440 445

Arg Phe Glu Arg Asn Thr Ser Asp Ala Phe Asn Lys Asn Leu Thr Met
450 455 460

Lys Asp Ala Ile Asn Met Thr Leu Ser Ile Ser Pro Glu Trp Leu Gln
465 470 475 480

Arg Arg Val His Glu Gln Tyr Ser Phe Gly Tyr Ser Lys Asn Glu Glu
485 490 495

Glu Leu Arg Lys Asn Glu Leu His His Lys His Trp Ser Asn Pro Met
500 505 510

Glu Val Pro Leu Pro Glu Ala Pro His Met Lys Ile Tyr Cys Ile Tyr
515 520 525

Gly Val Asn Asn Pro Thr Glu Arg Ala Tyr Val Tyr Lys Glu Glu Asp
530 535 540

Asp Ser Ser Ala Leu Asn Leu Thr Ile Asp Tyr Glu Ser Lys Gln Pro
545 550 555 560

Val Phe Leu Thr Glu Gly Asp Gly Thr Val Pro Leu Val Ala His Ser
565 570 575

Met Cys His Lys Trp Ala Gln Gly Ala Ser Pro Tyr Asn Pro Ala Gly
580 585 590

Ile Asn Val Thr Ile Val Glu Met Lys His Gln Pro Asp Arg Phe Asp
595 600 605

Ile Arg Gly Gly Ala Lys Ser Ala Glu His Val Asp Ile Leu Gly Ser
610 615 620

Ala Glu Leu Asn Asp Tyr Ile Leu Lys Ile Ala Ser Gly Asn Gly Asp
625 630 635 640

Leu Val Glu Pro Arg Gln Leu Ser Asn Leu Ser Gln Trp Val Ser Gln
645 650 655

Met Pro Phe Pro Met
660

<210> 2
<211> 387
<212> PRT
<213> Arabidopsis thaliana

<400> 2
Val Gly Ser Asn Val Tyr Pro Leu Ile Leu Val Pro Gly Asn Gly Gly
1 5 10 15

Asn Gln Leu Glu Val Arg Leu Asp Arg Glu Tyr Lys Pro Ser Ser Val
20 25 30

Trp Cys Ser Ser Trp Leu Tyr Pro Ile His Lys Lys Ser Gly Gly Trp
35 40 45

Phe Arg Leu Trp Phe Asp Ala Ala Val Leu Leu Ser Pro Phe Thr Arg
50 55 60

Cys Phe Ser Asp Arg Met Met Leu Tyr Tyr Asp Pro Asp Leu Asp Asp
65 70 75 80

Tyr Gln Asn Ala Pro Gly Val Gln Thr Arg Val Pro His Phe Gly Ser
85 90 95

Thr Lys Ser Leu Leu Tyr Leu Asp Pro Arg Leu Arg Asp Ala Thr Ser
100 105 110

Tyr Met Glu His Leu Val Lys Ala Leu Glu Lys Lys Cys Gly Tyr Val
115 120 125

Asn Asp Gln Thr Ile Leu Gly Ala Pro Tyr Asp Phe Arg Tyr Gly Leu
130 135 140

Ala Ala Ser Gly His Pro Ser Arg Val Ala Ser Gln Phe Leu Gln Asp
145 150 155 160

Leu Lys Gln Leu Val Glu Lys Thr Ser Ser Glu Asn Glu Gly Lys Pro
165 170 175

Val Ile Leu Leu Ser His Ser Leu Gly Gly Leu Phe Val Leu His Phe
180 185 190

Leu Asn Arg Thr Thr Pro Ser Trp Arg Arg Lys Tyr Ile Lys His Phe
195 200 205

Val Ala Leu Ala Ala Pro Trp Gly Gly Thr Ile Ser Gln Met Lys Thr
210 215 220

Phe Ala Ser Gly Asn Thr Leu Gly Val Pro Leu Val Asn Pro Leu Leu
225 230 235 240

Val Arg Arg His Gln Arg Thr Ser Glu Ser Asn Gln Trp Leu Leu Pro
245 250 255

Ser Thr Lys Val Phe His Asp Arg Thr Lys Pro Leu Val Val Thr Pro
260 265 270

Gln Val Asn Tyr Thr Ala Tyr Glu Met Asp Arg Phe Phe Ala Asp Ile
275 280 285

Gly Phe Ser Gln Gly Val Val Pro Tyr Lys Thr Arg Val Leu Pro Leu
290 295 300

Thr Glu Glu Leu Met Thr Pro Gly Val Pro Val Thr Cys Ile Tyr Gly
305 310 315 320

Arg Gly Val Asp Thr Pro Glu Val Leu Met Tyr Gly Lys Gly Gly Phe
325 330 335

Asp Lys Gln Pro Glu Ile Lys Tyr Gly Asp Gly Asp Gly Thr Val Asn
340 345 350

Leu Ala Ser Leu Ala Ala Leu Lys Val Asp Ser Leu Asn Thr Val Glu
355 360 365

Ile Asp Gly Val Ser His Thr Ser Ile Leu Lys Asp Glu Ile Ala Leu
370 375 380

Lys Glu Ile
385

<210> 30
<211> 389
<212> PRT
<213> Arabidopsis thaliana

<400> 3
Leu Lys Lys Glu Gly Leu Lys Ala Lys His Pro Val Val Phe Ile Pro
1 5 10 15

Gly Ile Val Thr Gly Gly Leu Glu Leu Trp Glu Gly Lys Gln Cys Ala
20 25 30

Asp Gly Leu Phe Arg Lys Arg Leu Trp Gly Gly Thr Phe Leu Cys Trp
35 40 45

Val Glu His Met Ser Leu Asp Asn Glu Thr Gly Leu Asp Pro Ala Gly
50 55 60

Ile Arg Val Arg Ala Val Ser Gly Leu Val Ala Ala Asp Tyr Phe Ala
65 70 75 80

Pro Gly Tyr Phe Val Trp Ala Val Leu Ile Ala Asn Leu Ala His Ile
85 90 95

Gly Tyr Glu Glu Lys Asn Met Tyr Met Ala Ala Tyr Asp Trp Arg Leu
100 105 110

Ser Phe Gln Asn Thr Glu Arg Asp Gln Thr Leu Ser Arg Met Lys Ser
115 120 125

Asn Ile Glu Leu Met Val Ser Thr Asn Gly Gly Lys Lys Ala Val Ile
130 135 140

Val Pro His Ser Met Gly Val Leu Tyr Phe Leu His Phe Met Lys Trp
145 150 155 160

Val Glu Ala Pro Ala Pro Leu Gly Gly Gly Gly Gly Pro Asp Trp Cys
165 170 175

Ala Lys Tyr Ile Lys Ala Val Met Asn Ile Gly Gly Pro Phe Leu Gly
180 185 190

Val Pro Lys Ala Val Ala Gly Leu Phe Ser Ala Glu Ala Lys Asp Met
195 200 205

Arg Met Thr Arg Thr Trp Asp Ser Thr Met Ser Met Leu Pro Lys Gly
210 215 220

Gly Asp Thr Ile Trp Gly Gly Leu Asp Trp Ser Pro Glu Leu Pro Asn
225 230 235 240

Ala Pro Glu Met Glu Ile Tyr Ser Leu Tyr Gly Val Gly Ile Pro Thr
245 250 255

Glu Arg Ala Tyr Val Tyr Lys Leu Asn Gln Ser Pro Asp Ser Cys Ile
260 265 270

Pro Phe Gln Ile Phe Thr Ser Ala His Glu Glu Asp Glu Asp Ser Cys
275 280 285

Leu Lys Ala Gly Val Tyr Asn Val Asp Gly Asp Glu Thr Val Pro Val
290 295 300

Leu Ser Ala Gly Tyr Met Cys Ala Lys Ala Trp Arg Gly Lys Thr Arg
305 310 315 320

Phe Asn Pro Ser Gly Ile Lys Thr Tyr Ile Arg Glu Tyr Asn His Ser
325 330 335

Pro Pro Ala Asn Leu Leu Glu Gly Arg Gly Thr Gln Ser Gly Ala His
340 345 350

Val Asp Ile Met Gly Asn Phe Ala Leu Ile Glu Asp Ile Met Arg Val

355 360 365

Ala Ala Gly Gly Asn Gly Ser Asp Ile Gly His Asp Gln Val His Ser
370 375 380

Gly Ile Phe Glu Trp
385

<210> 4a
<211> 1986
<212> DNA
<213> Saccharomyces cerevisiae

<220>
<221> CDS
<222> (1)..(1983)

<400> 4

```
atg ggc aca ctg ttt cga aga aat gtc cag aac caa aag agt gat tct   48
Met Gly Thr Leu Phe Arg Arg Asn Val Gln Asn Gln Lys Ser Asp Ser
 1               5                  10                  15

gat gaa aac aat aaa ggg ggt tct gtt cat aac aag cga gag agc aga   96
Asp Glu Asn Asn Lys Gly Gly Ser Val His Asn Lys Arg Glu Ser Arg
            20                  25                  30

aac cac att cat cat caa cag gga tta ggc cat aag aga aga agg ggt  144
Asn His Ile His His Gln Gln Gly Leu Gly His Lys Arg Arg Arg Gly
        35                  40                  45

att agt ggc agt gca aaa aga aat gag cgt ggc aaa gat ttc gac agg  192
Ile Ser Gly Ser Ala Lys Arg Asn Glu Arg Gly Lys Asp Phe Asp Arg
    50                  55                  60

aaa aga gac ggg aac ggt aga aaa cgt tgg aga gat tcc aga aga ctg  240
Lys Arg Asp Gly Asn Gly Arg Lys Arg Trp Arg Asp Ser Arg Arg Leu
65                  70                  75                  80

att ttc att ctt ggt gca ttc tta ggt gta ctt ttg ccg ttt agc ttt  288
Ile Phe Ile Leu Gly Ala Phe Leu Gly Val Leu Leu Pro Phe Ser Phe
                85                  90                  95

ggc gct tat cat gtt cat aat agc gat agc gac ttg ttt gac aac ttt  336
Gly Ala Tyr His Val His Asn Ser Asp Ser Asp Leu Phe Asp Asn Phe
            100                 105                 110

gta aat ttt gat tca ctt aaa gtg tat ttg gat gat tgg aaa gat gtt  384
Val Asn Phe Asp Ser Leu Lys Val Tyr Leu Asp Asp Trp Lys Asp Val
        115                 120                 125

ctc cca caa ggt ata agt tcg ttt att gat gat att cag gct ggt aac  432
Leu Pro Gln Gly Ile Ser Ser Phe Ile Asp Asp Ile Gln Ala Gly Asn
    130                 135                 140

tac tcc aca tct tct tta gat gat ctc agt gaa aat ttt gcc gtt ggt  480
Tyr Ser Thr Ser Ser Leu Asp Asp Leu Ser Glu Asn Phe Ala Val Gly
145                 150                 155                 160

aaa caa ctc tta cgt gat tat aat atc gag gcc aaa cat cct gtt gta  528
Lys Gln Leu Leu Arg Asp Tyr Asn Ile Glu Ala Lys His Pro Val Val
```

```
                    165                 170                 175

atg gtt cct ggt gtc att tct acg gga att gaa agc tgg gga gtt att   576
Met Val Pro Gly Val Ile Ser Thr Gly Ile Glu Ser Trp Gly Val Ile
                180                 185                 190

gga gac gat gag tgc gat agt tct gcg cat ttt cgt aaa cgg ctg tgg   624
Gly Asp Asp Glu Cys Asp Ser Ser Ala His Phe Arg Lys Arg Leu Trp
        195                 200                 205

gga agt ttt tac atg ctg aga aca atg gtt atg gat aaa gtt tgt tgg   672
Gly Ser Phe Tyr Met Leu Arg Thr Met Val Met Asp Lys Val Cys Trp
    210                 215                 220

ttg aaa cat gta atg tta gat cct gaa aca ggt ctg gac cca ccg aac   720
Leu Lys His Val Met Leu Asp Pro Glu Thr Gly Leu Asp Pro Pro Asn
225                 230                 235                 240

ttt acg cta cgt gca gca cag ggc ttc gaa tca act gat tat ttc atc   768
Phe Thr Leu Arg Ala Ala Gln Gly Phe Glu Ser Thr Asp Tyr Phe Ile
                245                 250                 255

gca ggg tat tgg att tgg aac aaa gtt ttc caa aat ctg gga gta att   816
Ala Gly Tyr Trp Ile Trp Asn Lys Val Phe Gln Asn Leu Gly Val Ile
            260                 265                 270

ggc tat gaa ccc aat aaa atg acg agt gct gcg tat gat tgg agg ctt   864
Gly Tyr Glu Pro Asn Lys Met Thr Ser Ala Ala Tyr Asp Trp Arg Leu
        275                 280                 285

gca tat tta gat cta gaa aga cgc gat agg tac ttt acg aag cta aag   912
Ala Tyr Leu Asp Leu Glu Arg Arg Asp Arg Tyr Phe Thr Lys Leu Lys
    290                 295                 300

gaa caa atc gaa ctg ttt cat caa ttg agt ggt gaa aaa gtt tgt tta   960
Glu Gln Ile Glu Leu Phe His Gln Leu Ser Gly Glu Lys Val Cys Leu
305                 310                 315                 320

att gga cat tct atg ggt tct cag att atc ttt tac ttt atg aaa tgg  1008
Ile Gly His Ser Met Gly Ser Gln Ile Ile Phe Tyr Phe Met Lys Trp
                325                 330                 335

gtc gag gct gaa ggc cct ctt tac ggt aat ggt ggt cgt ggc tgg gtt  1056
Val Glu Ala Glu Gly Pro Leu Tyr Gly Asn Gly Gly Arg Gly Trp Val
            340                 345                 350

aac gaa cac ata gat tca ttc att aat gca gca ggg acg ctt ctg ggc  1104
Asn Glu His Ile Asp Ser Phe Ile Asn Ala Ala Gly Thr Leu Leu Gly
        355                 360                 365

gct cca aag gca gtt cca gct cta att agt ggt gaa atg aaa gat acc  1152
Ala Pro Lys Ala Val Pro Ala Leu Ile Ser Gly Glu Met Lys Asp Thr
    370                 375                 380

att caa tta aat acg tta gcc atg tat ggt ttg gaa aag ttc ttc tca  1200
Ile Gln Leu Asn Thr Leu Ala Met Tyr Gly Leu Glu Lys Phe Phe Ser
385                 390                 395                 400

aga att gag aga gta aaa atg tta caa acg tgg ggt ggt ata cca tca  1248
Arg Ile Glu Arg Val Lys Met Leu Gln Thr Trp Gly Gly Ile Pro Ser
                405                 410                 415
```

```
atg cta cca aag gga gaa gag gtc att tgg ggg gat atg aag tca tct   1296
Met Leu Pro Lys Gly Glu Glu Val Ile Trp Gly Asp Met Lys Ser Ser
            420                 425                 430

tca gag gat gca ttg aat aac aac act gac aca tac ggc aat ttc att   1344
Ser Glu Asp Ala Leu Asn Asn Asn Thr Asp Thr Tyr Gly Asn Phe Ile
        435                 440                 445

cga ttt gaa agg aat acg agc gat gct ttc aac aaa aat ttg aca atg   1392
Arg Phe Glu Arg Asn Thr Ser Asp Ala Phe Asn Lys Asn Leu Thr Met
    450                 455                 460

aaa gac gcc att aac atg aca tta tcg ata tca cct gaa tgg ctc caa   1440
Lys Asp Ala Ile Asn Met Thr Leu Ser Ile Ser Pro Glu Trp Leu Gln
465                 470                 475                 480

aga aga gta cat gag cag tac tcg ttc ggc tat tcc aag aat gaa gaa   1488
Arg Arg Val His Glu Gln Tyr Ser Phe Gly Tyr Ser Lys Asn Glu Glu
                485                 490                 495

gag tta aga aaa aat gag cta cac cac aag cac tgg tcg aat cca atg   1536
Glu Leu Arg Lys Asn Glu Leu His His Lys His Trp Ser Asn Pro Met
            500                 505                 510

gaa gta cca ctt cca gaa gct ccc cac atg aaa atc tat tgt ata tac   1584
Glu Val Pro Leu Pro Glu Ala Pro His Met Lys Ile Tyr Cys Ile Tyr
        515                 520                 525

ggg gtg aac aac cca act gaa agg gca tat gta tat aag gaa gag gat   1632
Gly Val Asn Asn Pro Thr Glu Arg Ala Tyr Val Tyr Lys Glu Glu Asp
    530                 535                 540

gac tcc tct gct ctg aat ttg acc atc gac tac gaa agc aag caa cct   1680
Asp Ser Ser Ala Leu Asn Leu Thr Ile Asp Tyr Glu Ser Lys Gln Pro
545                 550                 555                 560

gta ttc ctc acc gag ggg gac gga acc gtt ccg ctc gtg gcg cat tca   1728
Val Phe Leu Thr Glu Gly Asp Gly Thr Val Pro Leu Val Ala His Ser
                565                 570                 575

atg tgt cac aaa tgg gcc cag ggt gct tca ccg tac aac cct gcc gga   1776
Met Cys His Lys Trp Ala Gln Gly Ala Ser Pro Tyr Asn Pro Ala Gly
            580                 585                 590

att aac gtt act att gtg gaa atg aaa cac cag cca gat cga ttt gat   1824
Ile Asn Val Thr Ile Val Glu Met Lys His Gln Pro Asp Arg Phe Asp
        595                 600                 605

ata cgt ggt gga gca aaa agc gcc gaa cac gta gac atc ctc ggc agc   1872
Ile Arg Gly Gly Ala Lys Ser Ala Glu His Val Asp Ile Leu Gly Ser
    610                 615                 620

gcg gag ttg aac gat tac atc ttg aaa att gca agc ggt aat ggc gat   1920
Ala Glu Leu Asn Asp Tyr Ile Leu Lys Ile Ala Ser Gly Asn Gly Asp
625                 630                 635                 640

ctc gtc gag cca cgc caa ttg tct aat ttg agc cag tgg gtt tct cag   1968
Leu Val Glu Pro Arg Gln Leu Ser Asn Leu Ser Gln Trp Val Ser Gln
                645                 650                 655
```

1986

atg ccc ttc cca atg taa
Met Pro Phe Pro Met
660

<210> 5
<211> 661
<212> PRT
<213> Saccharomyces cerevisiae

<400> 5
Met Gly Thr Leu Phe Arg Arg Asn Val Gln Asn Gln Lys Ser Asp Ser
1 5 10 15

Asp Glu Asn Asn Lys Gly Gly Ser Val His Asn Lys Arg Glu Ser Arg
20 25 30

Asn His Ile His His Gln Gln Gly Leu Gly His Lys Arg Arg Arg Gly
35 40 45

Ile Ser Gly Ser Ala Lys Arg Asn Glu Arg Gly Lys Asp Phe Asp Arg
50 55 60

Lys Arg Asp Gly Asn Gly Arg Lys Arg Trp Arg Asp Ser Arg Arg Leu
65 70 75 80

Ile Phe Ile Leu Gly Ala Phe Leu Gly Val Leu Leu Pro Phe Ser Phe
85 90 95

Gly Ala Tyr His Val His Asn Ser Asp Ser Asp Leu Phe Asp Asn Phe
100 105 110

Val Asn Phe Asp Ser Leu Lys Val Tyr Leu Asp Asp Trp Lys Asp Val
115 120 125

Leu Pro Gln Gly Ile Ser Ser Phe Ile Asp Asp Ile Gln Ala Gly Asn
130 135 140

Tyr Ser Thr Ser Ser Leu Asp Asp Leu Ser Glu Asn Phe Ala Val Gly
145 150 155 160

Lys Gln Leu Leu Arg Asp Tyr Asn Ile Glu Ala Lys His Pro Val Val
165 170 175

Met Val Pro Gly Val Ile Ser Thr Gly Ile Glu Ser Trp Gly Val Ile
180 185 190

Gly Asp Asp Glu Cys Asp Ser Ser Ala His Phe Arg Lys Arg Leu Trp
195 200 205

Gly Ser Phe Tyr Met Leu Arg Thr Met Val Met Asp Lys Val Cys Trp
210 215 220

Leu Lys His Val Met Leu Asp Pro Glu Thr Gly Leu Asp Pro Pro Asn
225 230 235 240

Phe Thr Leu Arg Ala Ala Gln Gly Phe Glu Ser Thr Asp Tyr Phe Ile
245 250 255

Ala Gly Tyr Trp Ile Trp Asn Lys Val Phe Gln Asn Leu Gly Val Ile
260 265 270

Gly Tyr Glu Pro Asn Lys Met Thr Ser Ala Ala Tyr Asp Trp Arg Leu
275 280 285

Ala Tyr Leu Asp Leu Glu Arg Arg Asp Arg Tyr Phe Thr Lys Leu Lys
290 295 300

Glu Gln Ile Glu Leu Phe His Gln Leu Ser Gly Glu Lys Val Cys Leu
305 310 315 320

Ile Gly His Ser Met Gly Ser Gln Ile Ile Phe Tyr Phe Met Lys Trp
325 330 335

Val Glu Ala Glu Gly Pro Leu Tyr Gly Asn Gly Gly Arg Gly Trp Val
340 345 350

Asn Glu His Ile Asp Ser Phe Ile Asn Ala Ala Gly Thr Leu Leu Gly
355 360 365

Ala Pro Lys Ala Val Pro Ala Leu Ile Ser Gly Glu Met Lys Asp Thr
370 375 380

Ile Gln Leu Asn Thr Leu Ala Met Tyr Gly Leu Glu Lys Phe Phe Ser
385 390 395 400

Arg Ile Glu Arg Val Lys Met Leu Gln Thr Trp Gly Gly Ile Pro Ser
405 410 415

Met Leu Pro Lys Gly Glu Glu Val Ile Trp Gly Asp Met Lys Ser Ser
420 425 430

Ser Glu Asp Ala Leu Asn Asn Asn Thr Asp Thr Tyr Gly Asn Phe Ile
435 440 445

Arg Phe Glu Arg Asn Thr Ser Asp Ala Phe Asn Lys Asn Leu Thr Met
450 455 460

Lys Asp Ala Ile Asn Met Thr Leu Ser Ile Ser Pro Glu Trp Leu Gln
465 470 475 480

Arg Arg Val His Glu Gln Tyr Ser Phe Gly Tyr Ser Lys Asn Glu Glu
485 490 495

Glu Leu Arg Lys Asn Glu Leu His His Lys His Trp Ser Asn Pro Met
500 505 510

Glu Val Pro Leu Pro Glu Ala Pro His Met Lys Ile Tyr Cys Ile Tyr
515 520 525

Gly Val Asn Asn Pro Thr Glu Arg Ala Tyr Val Tyr Lys Glu Glu Asp
530 535 540

Asp Ser Ser Ala Leu Asn Leu Thr Ile Asp Tyr Glu Ser Lys Gln Pro
545 550 555 560

Val Phe Leu Thr Glu Gly Asp Gly Thr Val Pro Leu Val Ala His Ser
565 570 575

Met Cys His Lys Trp Ala Gln Gly Ala Ser Pro Tyr Asn Pro Ala Gly
580 585 590

Ile Asn Val Thr Ile Val Glu Met Lys His Gln Pro Asp Arg Phe Asp
595 600 605

Ile Arg Gly Gly Ala Lys Ser Ala Glu His Val Asp Ile Leu Gly Ser
610 615 620

Ala Glu Leu Asn Asp Tyr Ile Leu Lys Ile Ala Ser Gly Asn Gly Asp
625 630 635 640

Leu Val Glu Pro Arg Gln Leu Ser Asn Leu Ser Gln Trp Val Ser Gln
645 650 655

Met Pro Phe Pro Met
660

SEQUENCE LISTING

<110> Stymne Dr., Sten
<120>

<130>
<140>
<141>
<160>

<210> 1
<211> 1986
<212> genomic DNA
<213> Saccharomyces cerevisiae
<220>
<221> CDS
<222> (1)..(1983)
<400> 1

atg ggc acc ctg ttt cga aga aat gtc cag aac caa aag agt gat tct 48
Met Gly Thr Leu Phe Arg Arg Asn Val Gln Asn Gln Lys Ser Asp Ser
1 5 10 15

gat gat aac aat aaa ggg ggt tct gtt cat aac aag cga gag agc aga 96
Asp Glu Asn Asn Lys Gly Gly Ser Val His Asn Lys Arg Glu Ser Arg
20 25 30

aac cac att cat cat caa cag gga tta ggc cat aag aga aga agg ggt 144
Asn His Ile His His Gln Gln Gly Leu Gly His Lys Arg Arg Arg Gly
35 40 45

att agt ggc agt gca aaa aga aat gag cgt ggc aaa gat ttc gac agg 192
Ile Ser Gly Ser Ala Lys Arg Asn Glu Arg Gly Lys Asp Phe Asp Arg
50 55 60

aaa aga gac ggg aac ggt aga aaa cgt tgg aga gat tcc aga aga ctg 240
Lys Arg Asp Gly Asn Gly Arg Lys Arg Trp Arg Asp Ser Arg Arg Leu
65 70 75 80

att ttc att ctt ggt gca ttc tta ggt gta ttt ttg ccg ttt agc ttt 288
Ile Phe Ile Leu Gly Ala Phe Leu Gly Val Leu Leu Pro Phe Ser Phe
85 90 95

ggc gct tat cat gtt cat aat agc gat agc gac ttg ttt gac aac ttt 336
Gly Ala Tyr His Val His Asn Ser Asp Ser Asp Leu Phe Asp Asn Phe
100 105 110

```
gta aat ttt gat tca ctt aaa gtg tat ttg gat gat tgg aaa gat gtt   384
Val Asn Phe Asp Ser Leu Lys Val Tyr Leu Asp Asp Trp Lys Asp Val
        115                 120                 125

ctc cca caa ggt ata agt tcg ttt att gat gat att cag gct ggt aac   432
Leu Pro Gln Gly Ile Ser Ser Phe Ile Asp Asp Ile Gln Ala Gly Asn
    130                 135                 140

tac tcc aca tct tct tta gat gat ctc agt gaa aat ttt gct gtt ggt   480
Tyr Ser Thr Ser Ser Leu Asp Asp Leu Ser Glu Asn Phe Ala Val Gly
145                 150                 155                 160

aaa caa ctc tta cgt gat tat aat atc gag gcc aaa cat [illegible]   528
Lys Gln Leu Leu Arg Asp Tyr Asn Ile Glu Ala Lys His Pro Val Val
                165                 170                 175

atg gtt cct ggt gtc att tct acg gga att gaa agc tgg gga gtt ttt   576
Met Val Pro Gly Val Ile Ser Thr Gly Ile Glu Ser Trp Gly Val Ile
            180                 185                 190

gga gac gat gag tgc gat agt [illegible] gag cat ttt cgt aaa cag ctg tgg   624
Gly Asp Asp Glu Cys Asp Ser Ser Ala His Phe Arg Lys Arg Leu Trp
        195                 200                 205

gga agt ttt tac atg ctg aga aca atg gtt atg gat aaa gtt tgt tgg   672
Gly Ser Phe Tyr Met Leu Arg Thr Met Val Met Asp Lys Val Cys Trp
    210                 215                 220

ttg aaa cat gta atg tta gat cct gaa aca ggt ctg gac cca aag aac   720
Leu Lys His Val Met Leu Asp Pro Glu Thr Gly Leu Asp Pro Pro Asn
225                 230                 235                 240

ttt acg cta cgt gca gca cag ggc ttc gaa tca act gat tat ttc atc   768
Phe Thr Leu Arg Ala Ala Gln Gly Phe Glu Ser Thr Asp Tyr Phe Ile
                245                 250                 255

gca ggg tat tgg att tgg aac aaa gtt ttc caa aat ctg gga gta att   816
Ala Gly Tyr Trp Ile Trp Asn Lys Val Phe Gln Asn Leu Gly Val Ile
            260                 265                 270

ggc tat gaa ccc aat aaa atg acg agt gct gcg tat gat tgg agg ctt   864
Gly Tyr Glu Pro Asn Lys Met Thr Ser Ala Ala Tyr Asp Trp Arg Leu
        275                 280                 285
```

```
gca tat tta gat cta gaa aga cgc gat agg tac ttt acg aag cta aag   912
Ala Tyr Leu Asp Leu Glu Arg Arg Asp Arg Tyr Phe Thr Lys Leu Lys
 290                 295                 300

gaa caa atc gaa ctg ttt cat caa ttg agt ggt gaa aaa gtt tgt tta   960
Glu Gln Ile Glu Leu Phe His Gln Leu Ser Gly Glu Lys Val Cys Leu
305                 310                 315                 320

att gga cat tct atg ggt tct cag att atc ttt tac ttt atg aaa tgg  1008
Ile Gly His Ser Met Gly Ser Gln Ile Ile Phe Tyr Phe Met Lys Trp
                325                 330                 335

gtc gag gct gaa ggc cct ctt tac ggt aat ggt ggt cgt ggc tgg gtt  1056
Val Glu Ala Glu Gly Pro Leu Tyr Gly Asn Gly Gly Arg Gly Trp Val
            340                 345                 350

aac gaa cac ata gat tca ttc att aat gca gca ggg acg ctt ctg ggc  1104
Asn Glu His Ile Asp Ser Phe Ile Asn Ala Ala Gly Thr Leu Leu Gly
        355                 360                 365

gct cca aag gca gtt cca gct cta att agt ggt gaa atg aaa gat acc  1152
Ala Pro Lys Ala Val Pro Ala Leu Ile Ser Gly Glu Met Lys Asp Thr
    370                 375                 380

att caa tta aat acg tta gcc atg tat ggt ttg gaa aag ttc ttc tca  1200
Ile Gln Leu Asn Thr Leu Ala Met Tyr Gly Leu Glu Lys Phe Phe Ser
385                 390                 395                 400

aga att gag aga gta aaa atg tta caa acg tgg ggt ggt att cca tca  1248
Arg Ile Glu Arg Val Lys Met Leu Gln Thr Trp Gly Gly Ile Pro Ser
                405                 410                 415

atg cta cca aag gga gaa gag gtc att tgg ggg gat atg aag tca tct  1296
Met Leu Pro Lys Gly Glu Glu Val Ile Trp Gly Asp Met Lys Ser Ser
            420                 425                 430

tca gag gat gca ttg aat aac aac act gac aca tac ggc aat ttc att  1344
Ser Glu Asp Ala Leu Asn Asn Asn Thr Asp Thr Tyr Gly Asn Phe Ile
        435                 440                 445

cga ttt gaa agg aat acg agc gat gct ttc aac aaa aat ttg aca atg  1392
Arg Phe Glu Arg Asn Thr Ser Asp Ala Phe Asn Lys Asn Leu Thr Met
    450                 455                 460

aaa gac gcc att aac atg aca tta tcg aaa tca cct gaa tgg ctc caa  1440
Lys Asp Ala Ile Asn Met Thr Leu Ser Ile Ser Pro Glu Trp Leu Gln
465                 470                 475                 480
```

```
aga agc gta cat gag cag tac tcg ttc ggc tat tcc aag aat gaa gaa   1488
Arg Arg Val His Glu Gln Tyr Ser Phe Gly Tyr Ser Lys Asn Glu Glu
                485                 490                 495

gag tta aga aaa aat gag cta cac cac aag cac tgg tcg aat cca atg   1536
Glu Leu Arg Lys Asn Glu Leu His His Lys His Trp Ser Asn Pro Met
            500                 505                 510

gaa gtt cca ctt cca gaa gct ccc cac atg aaa atc tat tgt ata tac   1584
Glu Val Pro Leu Pro Glu Ala Pro His Met Lys Ile Tyr Cys Ile Tyr
        515                 520                 525

ggg gtg aac aac cca act gaa agg gca tat gta tat aag gaa gag gat   1632
Gly Val Asn Asn Pro Thr Glu Arg Ala Tyr Val Tyr Lys Glu Glu Asp
    530                 535                 540

gac tcc tct gct ctg aat ttg acc atc gac tac gaa agc aag caa cct   1680
Asp Ser Ser Ala Leu Asn Leu Thr Ile Asp Tyr Glu Ser Lys Gln Pro
545                 550                 555                 560

gta ttc ctc acc gag ggg gac gga acc gtt ccg ctc gtg gcg cat tca   1728
Val Phe Leu Thr Glu Gly Asp Gly Thr Val Pro Leu Val Ala His Ser
                565                 570                 575

atg tgt cac aaa tgg gcc cag ggt gct tca ccg tac aac cct gcc gga   1776
Met Cys His Lys Trp Ala Gln Gly Ala Ser Pro Tyr Asn Pro Ala Gly
            580                 585                 590

att aac gtt act att gtg gaa atg aaa cac cag cca gat cga ttt gat   1824
Ile Asn Val Thr Ile Val Glu Met Lys His Gln Pro Asp Arg Phe Asp
        595                 600                 605

ata cgt ggt gga gca aaa agc gcc gaa cac gta gac atc ctc ggc agc   1872
Ile Arg Gly Gly Ala Lys Ser Ala Glu His Val Asp Ile Leu Gly Ser
    610                 615                 620

gcg gag ttg aac gat tac atc ttg aaa att gca agc ggt aat ggc gat   1920
Ala Glu Leu Asn Asp Tyr Ile Leu Lys Ile Ala Ser Gly Asn Gly Asp
625                 630                 635                 640

ctc gtc gag cca cgc caa ttg tct aat ttg agc cag tgg gtt tct cag   1968
Leu Val Glu Pro Arg Gln Leu Ser Asn Leu Ser Gln Trp Val Ser Gln
                645                 650                 655
```

SUBSTITUTE SHEET (RULE 26)

1986

atg ccc ttc cca atg taa
Met Pro Phe Pro Met
660

<210> 2 by
<211> 661
<212> PRT
<213> Saccharomyces cerevisiae

<400> 2

Met Gly Thr Leu Phe Arg Arg Asn Val Gln Asn Gln Lys Ser Asp Ser
1 5 10 15
Asp Glu Asn Asn Lys Gly Gly Ser Val His Asn Lys Arg Glu Ser Arg
20 25 30
Asn His Ile His His Gln Gln Gly Leu Gly His Lys Arg Arg Arg Gly
35 40 45
Ile Ser Gly Ser Ala Lys Arg Asn Glu Arg Gly Lys Asp Phe Asp Arg
50 55 60
Lys Arg Asp Gly Asn Gly Arg Lys Arg Trp Arg Asp Ser Arg Arg Leu
65 70 75 80
Ile Phe Ile Leu Gly Ala Phe Leu Gly Val Leu Leu Pro Phe Ser Phe
85 90 95
Gly Ala Tyr His Val His Asn Ser Asp Ser Asp Leu Phe Asp Asn Phe
100 105 110
Val Asn Phe Asp Ser Leu Lys Val Tyr Leu Asp Asp Trp Lys Asp Val
115 120 125
Leu Pro Gln Gly Ile Ser Ser Phe Ile Asp Asp Ile Gln Ala Gly Asn
130 135 140
Tyr Ser Thr Ser Ser Leu Asp Asp Leu Ser Glu Asn Phe Ala Val Gly
145 150 155 160
Lys Gln Leu Leu Arg Asp Tyr Asn Ile Glu Ala Lys His Pro Val Val
165 170 175
Met Val Pro Gly Val Ile Ser Thr Gly Ile Glu Ser Trp Gly Val Ile
180 185 190
Gly Asp Asp Glu Cys Asp Ser Ser Ala His Phe Arg Lys Arg Leu Trp
195 200 205
Gly Ser Phe Tyr Met Leu Arg Thr Met Val Met Asp Lys Val Cys Trp
210 215 220
Leu Lys His Val Met Leu Asp Pro Glu Thr Gly Leu Asp Pro Pro Asn
225 230 235 240
Phe Thr Leu Arg Ala Ala Gln Gly Phe Glu Ser Thr Asp Tyr Phe Ile
245 250 255
Ala Gly Tyr Trp Ile Trp Asn Lys Val Phe Gln Asn Leu Gly Val Ile
260 265 270
Gly Tyr Glu Pro Asn Lys Met Thr Ser Ala Ala Tyr Asp Trp Arg Leu
275 280 285
Ala Tyr Leu Asp Leu Glu Arg Arg Asp Arg Tyr Phe Thr Lys Leu Lys
290 295 300
Glu Gln Ile Glu Leu Phe His Gln Leu Ser Gly Glu Lys Val Cys Leu
305 310 315 320

Ile Gly His Ser Met Gly Ser Gln Ile Ile Phe Tyr Phe Met Lys Trp
325 330 335
Val Glu Ala Glu Gly Pro Leu Tyr Gly Asn Gly Gly Arg Gly Trp Val
340 345 350
Asn Glu His Ile Asp Ser Phe Ile Asn Ala Ala Gly Thr Leu Leu Gly
355 360 365
Ala Pro Lys Ala Val Pro Ala Leu Ile Ser Gly Glu Met Lys Asp Thr
370 375 380
Ile Gln Leu Asn Thr Leu Ala Met Tyr Gly Leu Glu Lys Phe Phe Ser
385 390 395 400
Arg Ile Glu Arg Val Lys Met Leu Gln Thr Trp Gly Gly Ile Pro Ser
405 410 415
Met Leu Pro Lys Gly Glu Glu Val Ile Trp Gly Asp Met Lys Ser Ser
420 425 430
Ser Glu Asp Ala Leu Asn Asn Asn Thr Asp Thr Tyr Gly Asn Phe Ile
435 440 445
Arg Phe Glu Arg Asn Thr Ser Asp Ala Phe Asn Lys Asn Leu Thr Met
450 455 460
Lys Asp Ala Ile Asn Met Thr Leu Ser Ile Ser Pro Glu Trp Leu Gln
465 470 475 480
Arg Arg Val His Glu Gln Tyr Ser Phe Gly Tyr Ser Lys Asn Glu Glu
485 490 495
Glu Leu Arg Lys Asn Glu Leu His His Lys His Trp Ser Asn Pro Met
500 505 510
Glu Val Pro Leu Pro Glu Ala Pro His Met Lys Ile Tyr Cys Ile Tyr
515 520 525
Gly Val Asn Asn Pro Thr Glu Arg Ala Tyr Val Tyr Lys Glu Glu Asp
530 535 540
Asp Ser Ser Ala Leu Asn Leu Thr Ile Asp Tyr Glu Ser Lys Gln Pro
545 550 555 560
Val Phe Leu Thr Glu Gly Asp Gly Thr Val Pro Leu Val Ala His Ser
565 570 575
Met Cys His Lys Trp Ala Gln Gly Ala Ser Pro Tyr Asn Pro Ala Gly
580 585 590
Ile Asn Val Thr Ile Val Glu Met Lys His Gln Pro Asp Arg Phe Asp
595 600 605
Ile Arg Gly Gly Ala Lys Ser Ala Glu His Val Asp Ile Leu Gly Ser
610 615 620
Ala Glu Leu Asn Asp Tyr Ile Leu Lys Ile Ala Ser Gly Asn Gly Asp
625 630 635 640
Leu Val Glu Pro Arg Gln Leu Ser Asn Leu Ser Gln Trp Val Ser Gln
645 650 655
Met Pro Phe Pro Met
660

<210> 3b
<211> 2312
<212> genomic DNA
<213> Schizosaccharomyces pombe
<400> 3

ATGGCGTCTT CCAAGAACAG CAAAACTCAT AAGAAAAAGA AAGAAGTCAA 50

ATCTCCTATC GACTTACCAA ATTCAAAGAA ACCAACTCGC GCTTTGAGTG 100

AGCAACCTTC AGCGTCCGAA ACACAATCTG TTTCAAATAA ATCAAGAAAA 150

TCTAAATTTG GAAAAAGATT GAATTTTATA TTGGGCGCTA TTTTGGGAAT 200

ATGCGGTGCT TTTTTTTTCG CTGTTCGAGA CGACAATGCT GTTTTCGACC 250

CTGCTACGTT AGATAAATTT GGGAATATGC TAGGCTCTTC AGACTTGTTT 300

GATCACATTA AAGGATATTT ATCTTATAAT GTGTTTAAGG ATGCACCTTT 350

TACTACGGAC AAGCCTTCGC AGTCTCCTAG CGGAAATGAA GTTCAAGTTG 400

GTCTTGATAT GTACAATGAG GGATATCGAA GTGACCATCC TGTTATTATG 450

GTTCCTGGTG TTATCAGCTC AGGATTAGAA AGTTGGTCGT TTAATAATTG 500

CTCGATTCCT TACTTTAGGA AACGTCTTTG GGGTAGCTGG TCTATGCTGA 550

AGGCAATGTT CCTTGACAAG CAATGCTGGC TTGAACATTT AATGCTTGAT 600

AAAAAAACCG GCTTGGATCC GAAGGGAATT AAGCTGCGAG CAGCTCAGGG 650

GTTTGAAGCA GCTGATTTTT TTATCACGGG CTATTGGATT TGGAGTAAAG 700

TAATTGAAAA CCTTGCTGCA ATTGGTTATG AGCCTAATAA CATGTTAAGT 750

GCTTCTTACG ATTGGCGGTT ATCATATGCA AATTTAGAGG AACGTGATAA 800

ATATTTTTCA AAGTTAAAAA TGTTCATTGA GTACAGCAAC ATTGTACATA 850

AGAAAAAGGT AGTGTTGATT TCTCACTCCA TGGGTTCACA GGTTACGTAC 900

TATTTTTTTA AGTGGGTTGA AGCTGAGGGC TACGGAAATG GTGGACCGAC 950

TTGGGTTAAT GATCATATTG AAGCATTTAT AAATGTGAGT CTCGATGGTT 1000

GTTTGACTAC GTTTCTAACT TTTGAATAGA TATCGGGATC TTTGATTGGA 1050

GCACCCAAAA CAGTGGCAGC GCTTTTATCG GGTGAAATGA AAGATACAGG 1100

TATTGTAATT ACATTAAACA TGTTAATATT TAATTTTTGC TAACCGTTTT 1150

AAGCTCAATT GAATCAGTTT TCGGTCTATG GGTAAGCAAT AAATTGTTGA 1200

GATTTGTTAC TAATTTACTG TTTAGTTTGG AAAAATTTTT TTCCCGTTCT 1250

GAGGTATATT CAAAAATACA AATGTGCTCT ACTTTTTCTA ACTTTTAATA 1300

GAGAGCCATG ATGGTTCGCA CTATGGGAGG AGTTAGTTCT ATGCTTCCTA 1350

AAGGAGGCGA TGTTGTATGG CGAAATGCCA GTTGGGTAAG AAATATGTGC 1400

TGTTAATTTT TTATTAATAT TTAGGCTCCA GATGATCTTA ATCAAACAAA 1450

TTTTTCCAAT GGTGCAATTA TTCGATATAG AGAAGACATT GATAAGGACC 1500

ACGATGAATT TGACATAGAT GATGCATTAC AATTTTTAAA AAATGTTACA 1550

GATGACGATT TTAAAGTCAT GCTAGCGAAA AATTATTCCC ACGGTCTTGC 1600

TTGGACTGAA AAAGAAGTGT TAAAAAATAA CGAAATGCCG TCTAAATGGA 1650

TAAATCCGCT AGAAGTAAGA ACATTAAAGT TACTAAATTA TACTAACCCA 1700

AATAGACTAG TCTTCCTTAT GCTCCTGATA TGAAAATTTA TTGCGTTCAC 1750

GGGGTCGGAA AACCAACTGA GAGAGGTTAT TATTATACTA ATAATCCTGA 1800

GGGGCAACCT GTCATTGATT CCTCGGTTAA TGATGGAACA AAAGTTGAAA 1850

ATGTGAGAGA ATTTATGTTT CAAACATTCT ATTAACTGTT TTATTAGGGT 1900

ATTGTTATGG ATGATGGTGA TGGAACTTTA CCAATATTAG CCCTTGGTTT 1950

GGTGTGCAAT AAAGTTTGGC AAACAAAAAG GTTTAATCCT GCTAATACAA 2000

GTATCACAAA TTATGAAATC AAGCATGAAC CTGCTGCGTT TGATCTGAGA 2050

GGAGGACCTC GCTCGGCAGA ACACGTCGAT ATACTTGGAC ATTCAGAGCT 2100

AAATGTATGT TCATTTTACC TTACAAATTT CTATTACTAA CTCTTGAAAT 2150

AAGGAAATTA TTTTAAAAGT TTCATCAGGC CATGGTGACT CGGTACCAAA 2200

CCGTTATATA TCAGATATCC AGTACGGACA TAAGTTTTGT AGATTGCAAT 2250

TAACTAACTA ACCGAACAGG GAAATAATAA ATGAGATAAA TCTCGATAAA 2300

CCTAGAAATT AA 2312

<210> 4b
<211> 3685
<212> genomic DNA
<213> Arabidopsis thaliana
<400> 4

ATGCCCCTTA TTCATCGGAA AAAGCCGACG GAGAAACCAT CGACGCCGCC 50
ATCTGAAGAG GTGGTGCACG ATGAGGATTC GCAAAAGAAA CCACACGAAT 100
CTTCCAAATC CCACCATAAG AAATCGAACG GAGGAGGGAA GTGGTCGTGC 150
ATCGATTCTT GTTGTTGGTT CATTGGGTGT GTGTGTGTAA CCTGGTGGTT 200
TCTTCTCTTC CTTTACAACG CAATGCCTGC GAGCTTCCCT CAGTATGTAA 250
CGGAGCGAAT CACGGGTCCT TTGCCTGACC CGCCCGGTGT TAAGCTCAAA 300
AAAGAAGGTC TTAAGGCGAA ACATCCTGTT GTCTTCATTC CTGGGATTGT 350
CACCGGTGGG CTCGAGCTTT GGGAAGGCAA ACAATGCGCT GATGGTTTAT 400
TTAGAAAACG TTTGTGGCGT GGAACTTTTG GTGAAGTCTA CAAAAGGTGA 450
GCTCAACAAT TCTCACTCTT CCTTTATATT GGGATTTGGA TTGGATCTGA 500
TGAGATCACG CACTTGTTGC TTCTTCAACA TCACTCAAAC TTTAATTCCA 550
TGTTTGTCTG TCTTACTCTT TACTTTTTTT TTTTTTTGAT GTGAAACGCT 600
ATTTTCTTAA GAGACTATTT CTGTATGTGT AAGGTAAGCG TTCCAAGGAC 650
GTAATTGGCT TGGACTATTT CTGTTTGATT GTTAACTTTA GGATATAAAA 700
TAGCTGCCTT GGAATTTCAA GTCATCTTAT TGCCAAATCT GTTGCTAGAC 750
ATGCCCTAGA GTCCGTTCAT AACAAGTTAC TTCCTTTACT GTCGTTGCGT 800
GTAGATTTAG CTTTGTGTAG CGTATAATGA AGTAGTGTTT TATGTTTTGT 850
TGGGAATAGA GAAGTTCTAA CTACATCTGT GGAAAGTGTG TTCAGGCTGT 900
GATAGAGGAC TGTTGCTTTA TTATTCAACT ATGTATATGT GTAATTAAAG 950
CTAGTTCCTT TTTGATCTTT CAGCTCAATG TGCTTTTCTC AATTTTTTTC 1000
TCAATTTCAA AGTTTCACAT CGAGTTTATT CACATGTCTT GAATTTCGTC 1050
CATCCTCGTT CTGTTATCCA GCTTTGAACT CCTCCCGACC CTGCTATGGA 1100
TATATTAAAA AAAAAGTGTT TTGTGGGTTG CATCTTTGTT ACGATCTGCA 1150
TCTTCTTCTT TCGGCTCAGT GTTCATGTTT TTGCTATGGT AGAGATGGGC 1200
AATGTTATTG TTGATGGTAA CAGTGGTATA GTTGATAGTA TCTTAACTAA 1250
TCAATTATCT CTTTGATTCA GGCCTCTATG TTGGGTGGAA CACATGTCAC 1300
TTGACAATGA AACTGGGTTG GATCCAGCTG GTATTAGAGT TCGAGCTGTA 1350

TCAGGACTCG TGGCTGCTGA CTACTTTGCT CCTGGCTACT TTGTCTGGGC 1400

AGTGCTGATT GCTAACCTTG CACATATTGG ATATGAAGAG AAAAATATGT 1450

ACATGGCTGC ATATGACTGG CGGCTTTCGT TTCAGAACAC AGAGGTTCTT 1500

TTCTCATCGT TCTTTCTATT ATTCTGTTCC ATGTTACGTT TCTTTCTTCA 1550

TTACTTAAGG CTTAAATATG TTTCATGTTG AATTAATAGG TACGTGATCA 1600

GACTCTTAGC CGTATGAAAA GTAATATAGA GTTGATGGTT TCTACCAACG 1650

GTGGAAAAAA AGCAGTTATA GTTCCGCATT CCATGGGGGT CTTGTATTTT 1700

CTACATTTTA TGAAGTGGGT TGAGGCACCA GCTCCTCTGG GTGGCGGGGG 1750

TGGGCCAGAT TGGTGTGCAA AGTATATTAA GGCGGTGATG AACATTGGTG 1800

GACCATTTCT TGGTGTTCCA AAAGCTGTTG CAGGGCTTTT CTCTGCTGAA 1850

GCAAAGGATG TTGCAGTTGC CAGGTATTGA ATATCTGCTT ATACTTTTGA 1900

TGATCAGAAC CTTCGCTCTG GAACTCAAAG TTATTCTACT AAATATCAAT 1950

TCTAATAACA TTGCTATATT ATCGCTGCAA CTGACATTGG TTGATTATTT 2000

TTGCTGCTTA TGTAACTGAA ACTCTCTTGA GATTAGACAA ATGATGAATT 2050

GATAATTCTT ACGCATTGCT CTGTGATGAC CAGTTTCTTA GCTTCGACGA 2100

TAACATTTGT CATACTGTCT TTTGGAGGGC ATTGAATTTT GCTATGGAAA 2150

GCGCTGGAGC TTCCATGCTT GCATTCTTTA CCAATTAGCG TTATTCTGCT 2200

TCTTTCAATT TTCTTGTATA TGCATCTATG GTCTTTTATT TCTTCTTAAT 2250

TAAAGACTCG TTGGATTAGT TGCTCTATTA GTCACTTGGT TCCTTAATAT 2300

AGAACTTTAC TTTCTTCGAA AATTGCAGAG CGATTGCCCC AGGATTCTTA 2350

GACACCGATA TATTTAGACT TCAGACCTTG CAGCATGTAA TGAGAATGAC 2400

ACGCACATGG GACTCAACAA TGTCTATGTT ACCGAAGGGA GGTGACACGA 2450

TATGGGGCGG GCTTGATTGG TCACCGGAGA AAGGCCACAC CTGTTGTGGG 2500

AAAAAGCAAA AGAACAACGA AACTTGTGGT GAAGCAGGTG AAAACGGAGT 2550

TTCCAAGAAA AGTCCTGTTA ACTATGGAAG GATGATATCT TTTGGGAAAG 2600

AAGTAGCAGA GGCTGCGCCA TCTGAGATTA ATAATATTGA TTTTCGAGTA 2650

AGGACATATA AATCATAATA AACCTTGTAC ATTTTGTGAT TGTATGATGA 2700

ATATCTGTAC ATTTTATCTG GTGAAGGGTG CTGTCAAAGG TCAGAGTATC 2750

CCAAATCACA CCTGTCGTGA CGTGTGGACA GAGTACCATG ACATGGGAAT 2800

TGCTGGGATC AAAGCTATCG CTGAGTATAA GGTCTACACT GCTGGTGAAG 2850

CTATAGATCT ACTACATTAT GTTGCTCCTA AGATGATGGC GCGTGGTGCC 2900

GCTCATTTCT CTTATGGAAT TGCTGATGAT TTGGATGACA CCAAGTATCA 2950

AGATCCCAAA TACTGGTCAA ATCCGTTAGA GACAAAGTAA GTGATTTCTT 3000

GATTCCAACT GTATCCTTCG TCCTGATGCA TTATCAGTCT TTTTGTTTTC 3050

GGTCTTGTTG GATATGGTTT TCAGCTCAAA GCTTACAAAG CTGTTTCTGA 3100

GCCTTTCTCA AAAAGGCTTG CTCAGTAATA TTGAGGTGCT AAAGTTGATA 3150

CATGTGACTC TTGCTTATAA ATCCTCCGTT TGGTTTGTTC TGCTTTTTCA 3200

GATTACCGAA TGCTCCTGAG ATGGAAATCT ACTCATTATA CGGAGTGGGG 3250

ATACCAACGG AACGAGCATA CGTATACAAG CTTAACCAGT CTCCCGACAG 3300

TTGCATCCCC TTTCAGATAT TCACTTCTGC TCACGAGGAG GACGAAGATA 3350

GCTGTCTGAA AGCAGGAGTT TACAATGTGG ATGCGGATGA AACAGTACCC 3400

GTCCTAAGTG CCGGGTACAT GTGTGCAAAA GCGTGGCGTG GCAAGACAAG 3450

ATTCAACCCT TCCGGAATCA AGACTTATAT AAGAGAATAC AATCACTCTC 3500

CGCCGGCTAA CCTGTTGGAA GGGCGCGGGA CGCAGAGTGG TGCCCATGTT 3550

GATATCATGG GAAACTTTGC TTTGATCGAA GATATCATGA GGGTTGCCGC 3600

CGGAGGTAAC GGGTCTGATA TAGGACATGA CCAGGTCCAC TCTGGCATAT 3650

TTGAATGGTC GGAGCGTATT GACCTGAAGC TGTGA 3685

<210> 5
<211> 402
<212> cDNA
<213> Arabidopsis thaliana
<220>
<221> CDS
<222> (120)..(402)
<400> 5

```
AGAAACAGCTCTTTGTCTCT CTCGACTGATCTAACAATCC CTAATCTGTGTTCTAAATTC    60

CTGGACGAGATTTGACAAAG TCCGTATAGCTTAACCTGGT TTAATTTCAAGTGACAGAT    119

ATG CCC CTT ATT CAT CGG AAA AAG CCG ACG CAG AAA CCA TCG ACG CCG   167
Met Pro Leu Ile His Arg Lys Lys Pro Thr Glu Lys Pro Ser Thr Pro

CCA TCT GAA GAG GTG GTG CAC GAT GAG GAT TCG CAA AAG AAA CCA CAC   215
Pro Ser Glu Glu Val Val His Asp Glu Asp Ser Gln Lys Lys Pro His

GAA TCT TCC AAA TCC CAC CAT AAG NAA TCG AAC GGA GGA GGG AAG TGG   263
Glu Ser Ser Lys Ser His His Lys ??? Ser Asn Gly Gly Gly Lys Trp

TCG TGC ATC GAT TCT TGT TGT TGG TTC ATT GGG TGT GTG TGT GTA ACC   311
Ser Cys Ile Asp Ser Cys Cys Trp Phe Ile Gly Cys Val Cys Val Thr

TGG TGG TTT CTT CTC TTC CTT TAC AAC GCA ATG CCT GCG AGC TTC CCT   359
Trp Trp Phe Leu Leu Phe Leu Tyr Asn Ala Met Pro Ala Ser Phe Pro

CAG TAT GTA ACG GAG CCG AAT CAC GNG TCC TTT GCC TTA CCC G         402
Gln Tyr Val Thr Glu Pro Asn His ??? Ser Phe Ala Leu Pro
```

<210> 6b
<211> 643
<212> cDNA
<213> Zea mays
<220>
<221> CDS
<222> (1)..(402)
<400> 6

```
CGG GAG AAA ATA GCT GCT TTG AAG GGG GGT GTT TAC TTA GCC GAT GGT   48
Arg Glu Lys Ile Ala Ala Leu Lys Gly Gly Val Tyr Leu Ala Asp Gly
  1               5                  10                  15

GAT GAA ACT GTT CCA GTT CTT AGT GCG GGC TAC ATG TGT GCG AAA GGA   96
Asp Glu Thr Val Pro Val Leu Ser Ala Gly Tyr Met Cys Ala Lys Gly
            20                  25                  30

TGG CGT GGC AAA ACT CGT TTC AGC CCT GCC GGC AGC AAG ACT TAC GTG  144
Trp Arg Gly Lys Thr Arg Phe Ser Pro Ala Gly Ser Lys Thr Tyr Val
        35                  40                  45

AGA GAA TAC AGC CAT TCG CCA CCC TCT ACT CTC CTG GAA GGC AGG GGC  192
Arg Glu Tyr Ser His Ser Pro Pro Ser Thr Leu Leu Glu Gly Arg Gly
    50                  55                  60

ACC CAG AGC GGT GCA CAT GTT GAT ATA ATG GGG AAC TTT GCT CTA ATT  240
Thr Gln Ser Gly Ala His Val Asp Ile Met Gly Asn Phe Ala Leu Ile
65                  70                  75                  80

GAG GAC GTC ATC AGA ATA GCT GCT GGG GCA ACC GGT GAG GAA ATT GGT  288
Glu Asp Val Ile Arg Ile Ala Ala Gly Ala Thr Gly Glu Glu Ile Gly
                85                  90                  95

GGC GAT CAG GTT TAT TCA GAT ATA TTC AAG TGG TCA GAG AAA ATC AAA  336
Gly Asp Gln Val Tyr Ser Asp Ile Phe Lys Trp Ser Glu Lys Ile Lys
            100                 105                 110

TTC AAA TTG TAA  CCTATGGGAA GTTAAAGAAG TGCCGACCCG TTTATTGCGTTCC  391

Leu Lys Leu
        115

AAAGTGTCCT GCCTGAGTGC AACTCTGGAT TTTGCTTAAA TATTGTAATT TTTCACGC   449

TTCATTCGTC CCTTTGTCAA ATTTACATTT GACAGGACGC CAATGCGATA CGATGTTG   507

TACCGCTATT TTCAGCATTG TATATTAAAC TGTACAGGTG TAAGTTGCAT TTGCCAGC   565

TGAAATTGTG TAGTCGTTTT CTTTACGATT TAATANCAAG TGGCGGAGCA GTGCCCCA   623

AGCNAAAAAA AAAAAAAAAA                                             643
```

<210> 7b
<211> 115
<212> PRT
<213> Zea mays
<400> 7

Arg Glu Lys Ile Ala Ala Leu Lys Gly Gly Val Tyr Leu Ala Asp Gly
1 5 10 15

Asp Glu Thr Val Pro Val Leu Ser Ala Gly Tyr Met Cys Ala Lys Gly
20 25 30

Trp Arg Gly Lys Thr Arg Phe Ser Pro Ala Gly Ser Lys Thr Tyr Val
35 40 45

Arg Glu Tyr Ser His Ser Pro Pro Ser Thr Leu Leu Glu Gly Arg Gly
50 55 60

Thr Gln Ser Gly Ala His Val Asp Ile Met Gly Asn Phe Ala Leu Ile
65 70 75 80

Glu Asp Val Ile Arg Ile Ala Ala Gly Ala Thr Gly Glu Glu Ile Gly
85 90 95

Gly Asp Gln Val Tyr Ser Asp Ile Phe Lys Trp Ser Glu Lys Ile Lys
100 105 110

Leu Lys Leu
115

<210> 8 *8*
<211> 616
<212> cDNA
<213> Neurospora crassa
<400> 8

GGTGGCGAAG ACGANGGCGG AAGTTGGAGG CTAACGAGAA TGACNCTCGG 50

AGATGGATCT ACCCTCTAGA GACACGACTA CCNTTGCACC CAGCCTCAAG 100

GTNTACNGTT TNTATGGGTA GGAAGCCGAC GGAGCGAGCC TACATCTATC 150

TGGCGCCCGA TCCCGGGACG ACAACGCATC TTTAGATGAC GATCGATACG 200

ACTTTGACTN AGGGCCACAT TGACCACGGT GTGATTTTGG GCGAAGGCGA 250

TGGCACAGTG AACCTTATGA GTTTGGGGTA CCTGTGCAAT AAGGGGTGGA 300

AAATGAACAG ATACAATCCT GCGGGCTCAA AAATAACCGT GGTCGAGATG 350

CCGCATGAAC CAGAACGGTT CAATCCGAGA GGAGGGCCGA ATACGGCGGA 500

CTTAAATATG TAGAAAAGGT TGAAATTTAT GAAGAGTAAT TAAATACGGC 550

ACATAGGTTA CTCAATAGTA TGACTAATTA AAAAAAAATT TTTTTTCTAA 600

AAAAAAAAAA AAAAAA 616

<210> 9
<211> 1562
<212> genomic DNA
<213> Arabidopsis thaliana
<400> 9

ATGAAAAAAA TATCTTCACA TTATTCGGTA GTCATAGCGA TACTCGTTGT 50

GGTGACGATG ACCTCGATGT GTCAAGCTGT GGGTAGCAAC GTGTACCCTT 100

TGATTCTGGT TCCAGGAAAC GGAGGTAACC AGCTAGAGGT ACGGCTGGAC 150

AGAGAATACA AGCCAAGTAG TGTCTGGTGT AGCAGCTGGT TATATCCGAT 200

TCATAAGAAG AGTGGTGGAT GGTTTAGGCT ATGGTTCGAT GCAGCAGTGT 250

TATTGTCTCC CTTCACCAGG TGCTTCAGCG ATCGAATGAT GTTGTACTAT 300

GACGCTGATT TGGATGATTA CCAAAATGCT CCTGGTGTCC AAACCCGGGT 350

TCCTCATTTC GGTTCGACCA AATCACTTCT ATACCTCGAC CCTCGTCTCC 400

GGTTAGTACT TTCCAAGATA TATCATTTTG GGACATTTGC ATAATGAACA 450

AAATAGACAT AAATTTGGGG GATTATTGTT ATATCAATAT CCATTTATAT 500

GCTAGTCGGT AATGTGAGTG TTATGTTAGT ATAGTTAATG TGAGTGTTAT 550

GTGATTTTCC ATTTTAAATG AAGCTAGAAA GTTGTCGTTT AATAATGTTG 600

CTATGTCATG AGAATTATAA GGACACTATG TAAATGTAGC TTAATAATAA 650

GGTTTGATTT GCAGAGATGC CACATCTTAC ATGGAACATT TGGTGAAAGC 700

TCTAGAGAAA AAATGCGGGT ATGTTAACGA CCAAACCATC CTAGGAGCTC 750

CATATGATTT CAGGTACGGC CTGGCTGCTT CGGGCCACCC GTCCCGTGTA 800

GCCTCACAGT TCCTACAAGA CCTCAAACAA TTGGTGGAAA AAACTAGCAG 850

CGAGAACGAA GGAAAGCCAG TGATACTCCT CTCCCATAGC CTAGGAGGAC 900

TTTTCGTCCT CCATTTCCTC AACCGTACCA CCCCTTCATG GCGCCGCAAG 950

TACATCAAAC ACTTTGTTGC ACTCGCTGCG CCATGGGGTG GGACGATCTC 1000

TCAGATGAAG ACATTTGCTT CTGGCAACAC ACTCGGTGTC CCTTTAGTTA 1050

ACCCTTTGCT GGTCAGACGG CATCAGAGGA CCTCCGAGAG TAACCAATGG 1100

CTACTTCCAT CTACCAAAGT GTTTCACGAC AGAACTAAAC CGCTTGTCGT 1150

AACTCCCCAG GTTAACTACA CAGCTTACGA GATGGATCGG TTTTTTGCAG 1200

ACATTGGATT CTCACAAGGA GTTGTGCCTT ACAAGACAAG AGTGTTGCCT 1250

TTAACAGAGG AGCTGATGAC TCCGGGAGTG CCAGTCACTT GCATATATGG 1300

GAGAGGAGTT GATACACCGG AGGTTTTGAT GTATGGAAAA GGAGGATTCG 1350

ATAAGCAACC AGAGATTAAG TATGGAGATG GAGATGGGAC GGTTAATTTG 1400

GCGAGCTTAG CAGCTTTGAA AGTCGATAGC TTGAACACCG TAGAGATTGA 1450

TGGAGTTTCG CATACATCTA TACTTAAAGA CGAGATCGCA CTTAAAGAGA 1500

TTATGAAGCA GATTTCAATT ATTAATTATG AATTAGCCAA TGTTAATGCC 1550

GTCAATGAAT GA 1562

<210> 10
<211> 3995
<212> genomic DNA
<213> Arabidopsis thaliana
<400> 10

ATGGGAGCGA ATTCGAAATC AGTAACGGCT TCCTTCACCG TCATCGCCGT 50
TTTTTTCTTG ATTTGCGGTG GCCGAACTGC GGTGGAGGAT GAGACCGAGT 100
TTCACGGCGA CTACTCGAAG CTATCGGGTA TAATCATTGC CCCAFTTCGG 150
TCGACGCAGC TACGAGCGTG GTCGATCCTT GACTGTCCAT ACACTCCGTT 200
CGACTTCAAT CCGCTCGACC TCGTATGGCT AGACACCACT AAGGTCCGTG 250
ATCTTCATTT CCTTCGCTCC TTATTCTGTC GGTCGAGTCA CTTGTTGATG 300
AATTCCAAGC GAAATATAGC AATGAAGCAT GTCTCGTCTC TCTTATTGAT 350
TCGTTCATTA GTCAACAGTG ACGCTTCTGA ATCTGAGTTT AGAGTCATAT 400
AAAACAGCTG ACTCGGCGAG TCTTTCCCAT CGCTTTTGGT TCGCTAAATG 450
TAGCGCAATG AATGTGTAAT TAGTCTGCGC TTTTTATTCA ACTAGATCTG 500
CAAGTTTTTC AGAGTGCTCA ATAGTAGTTA GAAAATGTTA GGTCATTTTA 550
CTTGTGCATT GTGATTCTTT TGGTTGTTGC TTACTGATCG ACGTGATGGA 600
TGGTTTACAG CTTCTTTCTG CTGTCAACTG CTGGTTTAAG TGTATGGTGC 650
TAGATCCTTA TAATCAAACA GACCATCCCG AGTGTAAGTC ACGGCCTGAC 700
AGTGGTCTTT CAGCCATCAC AGAATTGGAT CCAGGTTACA TAACAGGTAG 750
TTTCGGATTT TTCTTTCTTT TGAGTTTTCT TCAATTTGAT ATCATCTTGT 800
TGTGATATAA TATGGCTAAG TTCATTAATT TGGTCAATTT TCAGGTCCTC 850
TTTCTACTGT CTGGAAAGAG TGGCTTAAGT GGTGTGTTGA GTTTGGTATA 900
GAAGCAAATG CAATTGTCGC TGTTCCATAC GATTGGAGAT TGTCACCAAC 950
CAATTGGAA GAGCGTGACC TTTACTTTCA CAAGCTCAAG TTAGTCCTTA 1000
TCAGGCTAAT GTCTTTTATC TTCTCTTTTT ATGTAAGATA AGCTAAGAGC 1050
TCTGGTCGTC TTCCTTTTTG CAGGTTGACC TTTGAAACTG CTTTAAAACT 1100
CCGTGGCGGC CCTTCTATAG TATTTGCCCA TTCAATGGGT AATAATGTCT 1150
TCAGATACTT TCTGGAATGG CTGAGGCTAG AAATTGCACC AAAACATTAT 1200
TTGAACTGGC TTGATCAGCA TATCCATGCT TATTTCGCTG TTGGTACCGG 1250
CCTACTATCC TTAAGTTACC ATTTTATTTT TTCTCTAATT GGGGGAGTTA 1300
TGTTGTGACT TACTGGATTG AGCTCGATAC CTGATTTGTT GTTGATTTAG 1350
GAGCTCCTCT TCTTGGTTCT GTTGAGGCAA TCAAATCTAC TCTCTCTGGT 1400
GTAACGTTTG GCCTTCCTGT TTCTGAGGTG ACCTCTGACT TCTCTTTAGT 1450
TTTAAGTAGT TGATATCAAC CAGCTCTTAT AACTCACTGG ATTTTCCTTT 1500
TGAAAGTATT ACTTTTGTTA ATTGAACTGC TGTACGCGAT ATGGTATCTG 1550
TAGATCTTGA AGTGCTAGTT ATCAAAGAAC ATATTGTGGG TAGTATACCT 1600
GTCAGCGGCC TTAGCTAATA CAACCAAACC ACATGTACAC TGATTTAGTT 1650
TTCAGATTAT TATGGTAGAC TTTAAGTTGA GAAGAAACTT TGACTGAAAT 1700
CTTTTTATTT TAATAGGCTA TGATTTGTTT ATTGAAATCA TGTGACATAT 1750
TGACATGCGC TTCTCATGTT TTTTGTTGGC AAGGCTTCAG GGAACTGCTC 1800
GGTTGTTGTC CAATTCTTTT GCGTCGTCAT TGTGGCTTAT GCCATTTTCA 1850
AAGAATTGCA AGGGTGATAA CACATTCTGG ACGCATTTTT CTGGGGGTGC 1900
TGCAAAGAAA GATAAGCGCG TATACCACTG TGATGAAGAG GAATATCAAT 1950
CAAAATATTC TGGCTGGCCG ACAAATATTA TTAACATTGA AATTCCTTCC 200
ACTAGCGGTT AGACTCTGTA TATGCAACTG TAACACTAAC AAAAGTTTCA 2050
CCAAGAATGT TCACTCTCAT ATTTCGTTCC TTTGATGTGT ATCCATCAGT 2100
TACAGAAACA GCTCTAGTCA ACATGACCAG CATGGAATGT GGCCTTCCCA 2150
CCCTTTTGTC TTTCACAGCC CGTGAACTAG CAGATGGGAC TCTTTTCAAA 2200
GCAATAGAAG ACTATGACCC AGATAGCAAG AGGATGTTAC ACCAGTTAAA 2250
GAAGTACGTA CCTTTCTTTG TGATAAGAAA TATTGCTCAT CGATCATCAC 2300
TTGCTGGCTT CTTGTACGTC AAATTGTTTT GTTTAAATCT CTATATCAAT 2350
TGTTCATATG CTTTGTCTTT CTTACTATAA GAAACAAGTA TAATCAGAAA 2400
CCTTATTATT GATTATCAGT TCTCTCCTTA TATTATGGAA TGTCTTTTTC 2450
GTTTACAGTT ATGAATGCAA AAGGGGGTAT TTTAGTTGAT TGATTCTCTC 2500
ATTCTCTAGT TTGTTTTGAC TAATAGCGTC AATTTTGTTT TTCTAGCAAA 2550
TCTTTGTGAA TTATATATAA CATGCTAACT ATACTTTTCA GGTTGTATCA 2600
TGATGACCCT GTTTTTAATC CTCTGACTCC TTGGGAGAGA CCACCTATAA 2650
AAAATGTATT TTGCATATAT GGTGCTCATC TAAAGACAGA GGTATGATGC 2700
ATTCTCAATA TCACATTATG CGTTGACTTT GTTATTATAT TCCCCATTTG 2750

```
GTTTGCAATA TCTTTTTGAA TTATGATTTA TCTTCTCCCT TGCATCTTAT      2800
CCTATTAAGC GTTAAAGGTA CTAAATGTAT GAAGCTGTCT GTCATAGGTT      2850
GGTTATTACT TTGCCCCAAG TGGCAAACCT TATCCTGATA ATTGGATCAT      2900
CACGGATATC ATTTATGAAA CTGAAGGTTC CCTCGTGTCA AGGTAATTTT      2950
CCGCAATGGC AGAAGTAAAA CAGGAAGGCA AAGTCTTCTG TATCAGTCTA      3000
GTGGCATGTT ATCTCAGTTG CATAAGCAAA TTATTAAACA ACTAAAATTT      3050
AAGTACTTTT TTATCATTCC TTTTGAGCTT AGTGGATGAT CAGTGGCTTA      3100
AAGTGGGAAG AGGTGTTGCA TGAAACATGA CACTTGTATC AAAGATAACT      3150
ACCAAAACAA AACTAACCCA TTTCTGAATT TCATATTATT AGGAGTAGTC      3200
GTGCTTTTAA AAAATTTGTT TTAAGAAACC GAAAAACTAG TTCATATCTT      3250
GATTGTGCAA TATCTGCAGG TCTGGAACTG TGGTTGATGG GAACGCTGGA      3300
CCTATAACTG GGGATGAGAC GGTAAGCTCA GAAGTTGGTT TTGAAATTAT      3350
CTTCTTGCAA ACTACTGAAG ACTAAGATAA TACTTGCTTC TGGAACACTG      3400
CTTGCTATGT TCTCTAGTAC ACTGCAATAT TGACTCTCCG CTACTTTTAT      3450
TGATTATGAA ATTGATCTCT TATAGGTACC CTATCATTCA CTCTCTTGGT      3500
GCAAGAATTG GCTCGGACCT AAAGTTAACA TAACAATGGC TCCCCAGGTA      3550
CTCTTTTTTA GTTCCTCACC TTATATAGAT CAAACTTTAA GTGTACTTTT      3600
CTGGTTATGT GTTGATTTAC CTCCAATTTG TTCTTTCTAA AAATCATATA      3650
TCTCTGTACT CCTCAAGAAC TTGTATTAAT CTAAACGAGA TTCTCATTGG      3700
GAAAATAAAA CAACAGCCAG AACACGATGG AAGCGACGTA CATGTGGAAC      3750
TAAATGTTGA TCATGAGCAT GGGTCAGACA TCATAGCTAA CATGACAAAA      3800
GCACCAAGGG TTAAGTACAT AACCTTTTAT GAAGACTCTG AGAGCATTCC      3850
GGGGAAGAGA ACCGCAGTCT CGGAGCTTGA TAAAAGTGGG TATTAA         3896
```

<210> 11b
<211> 709
<212> cDNA
<213> tomato
<400> 11

CTGGGGCCAA AAGTGAACAT AACAAGGACA CCACAGTCAG AGCATGATGT 50

TCAGATGTAC AAGTGCATCT AAATATAGAG CATCAACATG GTGAAGATAT 100

CATTCCCAAT ATGACAAAGT TACCTACAAT GAAGTACATA ACCTATTATG 150

AGGATTCTCA AAGTTTTCCA GGGACAAGAA CAGCAGTTTG GGAGCTTGAT 200

AAAGCAAATC ACAGGAACAT TGTCAGATCT CCAGCTTTGA TGCGGGAGCT 250

GTGGCTTGAG ATGTGGCATG ATATTCATCC TGATAAAAAG TCCAAGTTTG 300

TTACAAAAGG TGGTGTCTGA TCCTCACTAT TTTCTTCTAT AAATGTTTGA 350

GTTTGTATTG ACATTGTAAG TATTGCAACA AAAAGCAAAG CGTGGGCCTC 400

TGAGGGATGA GGACTGCTAT TGGGATTACG GGAAAGCTCG ATGTGCATGG 450

GCTGAACATT GTGAATACAG GTTAGAATAT TCAAATTATA TTTTGCAAAA 500

TATTCTCTTT TTGTGTATTT AGGCCACCTT TCCCCGGTCA CAACGATGCA 550

GATATGTATT CGGGGATGTT CACCTGGGAC AGAGTTGCAG ATTGAAGAGT 600

TCTACAICTC ACATCCTGTC ACACTATGTG TGATATTTAA GAAACTTTGT 650

TTGGCGGAAC AACAAGTTTG CACAAACATT TGAAGAAGAA AGCGAAATGA 700

TTCAGAGAG 709

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 October 2000 (12.10.2000)

PCT

(10) International Publication Number
WO 00/60095 A3

(51) International Patent Classification[7]: C12N 15/54, 9/10, 15/81, 15/82, 1/16, 5/10, A01K 67/027, C12P 7/64

(21) International Application Number: PCT/EP00/02701

(22) International Filing Date: 28 March 2000 (28.03.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

99106656.4	1 April 1999 (01.04.1999)	EP
99111321.8	10 June 1999 (10.06.1999)	EP
60/180,687	7 February 2000 (07.02.2000)	US

(71) Applicant *(for all designated States except US)*: **BASF PLANT SCIENCE GMBH** [DE/DE]; D-67056 Ludwigshafen (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **DAHLQVIST, Anders** [SE/SE]; Hemmansvägen 2, S-244 66 Furulund (SE). **STAHL, Ulf** [SE/SE]; Liljegatan 7b, S-753 24 Uppsala (SE). **LENMAN, Marit** [SE/SE]; Revingegatan 13a, S-223 59 Lund (SE). **BANAS, Antoni** [SE/PL]; Wiolinowa 14, PL-08110 Siedlce (PL). **RONNE, Hans** [SE/SE]; Dirigentvägen 169, S-756 54 Uppsala (SE). **STYMNE, Sten** [SE/SE]; Torrlösa 1380, S-269 90 Svalöv (SE).

(74) Agent: **FITZNER, Uwe**; Lintorfer Str. 10, D-40878 Ratingen (DE).

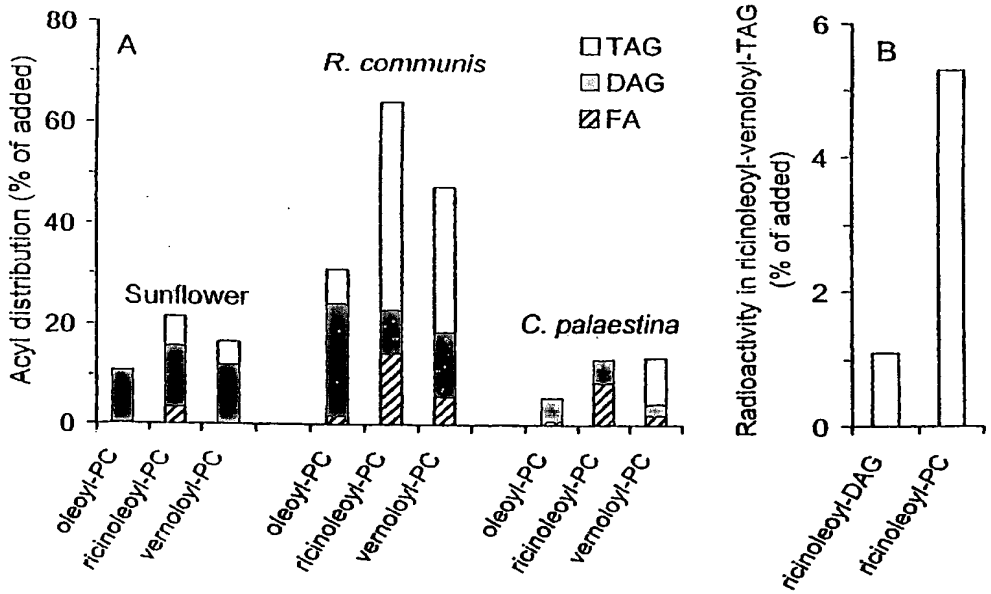
(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

[Continued on next page]

(54) Title: ENZYMES OF THE BIOSYNTHETIC PATHWAY FOR THE PRODUCTION OF TRIACYLGLYCEROL AND RECOMBINANT DNA MOLECULES ENCODING THESE ENZYMES



(57) Abstract: The present invention relates to the isolation, identification and characterization of nucleotide sequences encoding an enzyme catalysing the transfer of fatty acids from phospholipids to diacylglycerol in the biosynthetic pathway for the production of triacylglycerol, to the said enzymes and a process for the production of triacylglycerols.

WO 00/60095 A3

(88) Date of publication of the international search report:
1 February 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/02701

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/54 C12N9/10 C12N15/81 C12N15/82 C12N1/16
C12N5/10 A01K67/027 C12P7/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A01K C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
STRAND, EPO-Internal, WPI Data, MEDLINE, CHEM ABS Data, BIOSIS, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PETER VERHASSELT ET AL.: "Twelve open reading frames revealed in the 23.6kb segent flanking the centromere on the Saccharomyces cerevisiae chromosome XIV right arm" YEAST, vol. 10, no. 7, July 1994 (1994-07), pages 1355-1361, XP002112572 abstract; table 2	1-23,27
X	-& Swissprot Database Entry Yn84_Yeast Accession number P40345; 1 February 1995 XP002112574 the whole document	1-23,27

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 October 2000	30/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Montero Lopez, B

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! Database Entry SPBC776, 21 January 1999 (1999-01-21) LYNE M. ET AL.: "S. pombe chromosome II cosmid c776" Database accession no. AL035263 XP002150203 the whole document	1-23,27
X	DATABASE EMBL 'Online! Database Entry AI398644, 10 February 1999 (1999-02-10) XP002150204 the whole document & MARY ANNE NELSON ET AL.: "Expressed sequences from conidial, mycelial, and sexual stages of Neurospora crassa " FUNGAL GENETICS AND BIOLOGY, vol. 21, 1997, pages 348-363, XP000952173	1-23,27
X	KEITH STOBART ET AL.: "Triacylglycerols are synthesized and utilized by transacylation reactions in microsomal preparations of developing safflower (Carthamus tinctorius L.) seeds" PLANTA, vol. 203, no. 1, 1997, pages 58-66, XP002112573 page 58, right-hand column, last paragraph -page 59, left-hand column, paragraph 1 page 63, right-hand column, paragraph 2	25
A	WO 98 55631 A (CALGENE LLC) 10 December 1998 (1998-12-10) page 9, line 36 -page 10, line 7 page 12, line 28 -page 13, line 18 page 14, line 34 -page 15, line 13 page 20, line 5 -page 25, line 4	1-27
P,X	DATABASE SWALL 'Online! Database Entry 094680, 1 May 1999 (1999-05-01) LYNE M. ET AL.: "hypothetical 69.7 kDa protein C776.14 in chromosome II" Database accession no. 094680 XP002150205 the whole document	1-23,27

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/02701

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9855631 A	10-12-1998	CN 1266460 T	13-09-2000
		EP 1003882 A	31-05-2000